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NOVEL PEPTIDES AND PRODUCTION AND USE THEREOF

DECLARATION

I, Masashi Shimbo, technical translator, declare that I am a citizen of Japan, residing at c/o Takeda Chemical Industries, Ltd., of 17-85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka, Japan; that I am competent to make English translations and have had considerable experience in that work; that the attached are true translations into the English language of the Japanese Patent Application No. 146052/1996.

I, the undersigned, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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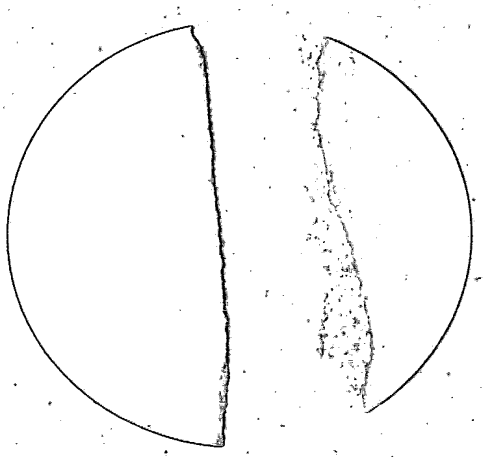

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[Document] Drawings 1

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[Title of the Invention] NOVEL PEPTIDES AND THEIR DNA

[Claims]

[Claim 1] A peptide comprising the amino acid sequence defined under SEQ ID NO:1, a precursor thereof, or a salt of said peptide or precursor.

[Claim 2] A precursor as claimed in Claim 1 which comprises the amino acid sequence defined under SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6.

[Claim 3] A peptide or precursor as claimed in Claim 1 which has somatostatin-like or cortistatin-like activity.

[Claim 4] A DNA comprising a DNA having a nucleotide sequence coding for the peptide or precursor claimed in Claim 1.

[Claim 5] A DNA as claimed in Claim 4 which comprises the nucleotide sequence defined under SEQ ID NO:7.

[Claim 6] A DNA as claimed in Claim 4 which comprises the nucleotide sequence defined under any of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12.

[Claim 7] A recombinant vector comprising the DNA claimed in Claim 4.

[Claim 8] A transformant harboring the recombinant vector claimed in Claim 7.

[Claim 9] A method of producing the peptide, precursor or salt claimed in Claim 1 which comprises growing the transformant claimed in Claim 8 to thereby cause production and accumulation of the peptide, precursor or salt claimed in Claim 1 and

harvesting the same.

[Claim 10] A pharmaceutical composition which comprises the peptide, precursor or salt claimed in Claim 1.

[Claim 11] A pharmaceutical composition comprising the DNA claimed in Claim 4.

[Claim 12] A pharmaceutical composition as claimed in Claim 10 or 11 which is an agent for the treatment or prevention of hormone-producing tumors, acromegaly, giantism, dementia or, a hormone secretion inhibitor, a tumor growth inhibitor, or a neural activity or sleep modulator.

[Claim 13] An antibody against the peptide, precursor or salt claimed in Claim 1.

[Claim 14] A method of screening for an antagonist or an agonist against a receptor for the peptide which comprise the peptide, precursor or salt as claimed in Claim 1.

[Claim 15] A kit for screening for an antagonist or an agonist against a receptor for the peptide, which kit comprises the peptide, precursor or salt as claimed in Claim 1.

[Detailed Description of the Invention]

[Technical Field to which the Invention belongs]

The present invention relates to novel physiologically active peptides, particularly peptides having human somatostatin-like or cortistatin-like activity, and precursors thereof.

[Prior Art]

Somatostatin was isolated from ovine hypothalamus and

identified as a growth hormone inhibiting factor (Guillemin, R. et al., Science, vol. 179, pp. 77-79, 1973). Somatostatin is composed of 14 amino acid residues and has a cyclic structure resulting from the S-S bond between Cys in position 3 and Cys in position 14 (somatostatin-14). Somatostatin-28, which is composed of somatostatin-14 and 14 amino acid residues added to the N-terminus of the somatostatin-14 molecule, has also been identified.

Somatostatin is broadly distributed in the central nervous system and, peripherally, occurs in such organs as the spleen and gastrointestinal tract, and further in the peripheral nerves. It is now known that this substance inhibits not only secretion of growth hormone but also secretion of pituitary hormones such as thyroid-stimulating hormone and prolactin and digestive tract hormones such as gastrin and insulin and that it also acts as a neurotransmitter (Brownstain, M. et al., Endocrinology, vol. 96, pp. 1456-1461, 1975). Furthermore, it has been found to inhibit cell proliferation. Therefore, various derivatives of somatostatin have been synthesized and tried for clinical application for the purpose of inhibiting hormone hypersecretion or tumor growth.

A novel neuropeptide similar in structure to somatostatin has been reported by a team of researchers at Scrips Laboratories. It has been revealed that this peptide named rat cortistatin (the precursor thereof being referred to as preprocortistatin) is the product of a gene different from the somatostatin gene. However, cortistatin has the property to selectively shorten the REM (rapid eye movement) sleep phase during sleep and generate low-frequency

waves in the cerebral cortex. Further, cortistatin impedes the effects of acetylcholine, which is itself a REM sleep inducer. Cortistatin may act as a modulator of neural activities and sleep (L. de Lecea et al., Nature, 381, 16 May 1996).

The activities of somatostatin depend on its binding to the specific high-affinity receptors (somatostatin receptors) present on the cell membrane and the consequent transduction of its signal through the GTP-binding protein to the intracellular signal transduction system. First, the structure of somatostatin receptor subtype 1 (hereinafter sometimes referred to as SSTR1) and that of subtype 2 (hereinafter sometimes referred to as SSTR2) were determined and reported (Yamada et al., Proc. Natl. Acad. Sci. USA, vol. 89, pp. 251-255, 1992). Then, DNAs coding for subtype 3 (hereinafter sometimes referred to as SSTR3), subtype 4 (hereinafter sometimes referred to as SSTR4) and subtype 5 (hereinafter sometimes referred to as SSTR5), respectively, were cloned (SSTR3: Yamada et al., Molecular Endocrinology, vol. 6, pp. 2136-2142, 1992; SSTR4 and SSTR5: Yamada et al., Biochem. Biophys. Res. Commun., vol. 195, pp. 844-852, 1993). These so-far known five somatostatin receptor subtypes are 42-60% homologous with one another on the amino acid level.

The activities of cortistatin are also supposedly displayed upon its binding to the specific high-affinity receptors on the cell membrane and the consequent transduction of its signal through the GTP-binding protein to the intracellular signal transduction system. In fact, cortistatin-14 undergoes a displacement similar to that

of somatostatin in response to the binding of [^{125}I]-labeled somatostatin on the membrane of the rat pituitary cell GH4 (L. de Lecea et al., Nature, 381, 16 May 1996). However, a possible difference in effect, for example on sleep, has been suggested between somatostatin-14 and cortistatin-14 intraventricularly administered to rats, and differences in affinity and site of action have been implied between the respective peptides with respect to somatostatin receptor subtypes and somatostatin receptor-like receptors. Furthermore, the probability has been pointed out that cortistatin also acts on receptors other than somatostatin receptors. For instance, GPR7 (U22491) and GPR8 (U22492) are reported to be receptors with high homology to somatostatin receptors although the binding thereof to somatostatin has not been established as yet [Genomics, 28, 84-91, (1995)]. It is considered possible that cortistatin act on such receptors as well. As mentioned above, cortistatin supposedly plays important roles in the regulation of physiological functions in vivo via specific receptors but no human-related somatostatin-like or cortistatin-like peptides are known as yet.

Attempts have been reportedly made to determine gene expression levels or discover novel genes in organs and cells by determining partial sequences (expressed sequence tags; abbreviated as ESTs) of cDNA clones randomly selected from among cDNA libraries. M. D. Adams et al. have reported a number of ESTs obtained from a brain cDNA library (Nature Genetics, vol. 4, pp. 373-380, 1993). Among the human ESTs of HGS (Human Genomu Science Inc.), HGS289122 and 1330029 showed high homology with the sequence of the rat prepro

cortidtatin (U51919) mentioned-above, in the result of homology search. However, the sequences of the ESTs are not accurate and only show a part of the nucleotode sequence. Therfore, it is not clear whether the gene which has the same nucleotide sequence of that of registered in the Database, is exist and has a function in the body.

The novel human derived physiologically active peptides are expected to enable development of novel drugs of value in the prevention or treatment of acute bacterial meningitis, acute myocardial infarction, acute pancreatitis, acute viral encephalitis, adult respiratory distress syndrome, alcoholic hepatitis, Alzheimer's disease, asthma, arteriosclerosis, atopic dermatitis, bacterial pneumonia, bladder cancer, bone fracture, mammary cancer, hyperphagia, polyphagia, burn healing, carcinoma of the uterine cervix, chronic lymphatic leukemia, chronic myelocytic leukemia, chronic pancreatitis, hepatic cirrhosis, colorectal cancer (carcinoma of the colon/rectum), Crohn's disease, dementia, diabetic complications, e.g. diabetic nephropathy, diabetic neuropathy, diabetic retinopathy, etc., gastritis, Helicobacter pylori infection, hepatic insufficiency, hepatitis A, hepatitis B, hepatitis C, other types of hepatitis, herpes simplex virus infection, varicella-zoster virus infection, Hodgkin's disease, AIDS virus infection, human papilloma virus infection, hypercalcemia, hypercholesterolemia, hyperglyceridemia, hyperlipemia, miscellaneous infectious diseases, influenza virus infection, insulin-dependent diabetes melitus (type I), invasive staphylococcal infection, malignant melanoma, cancer metastasis, multiple myeloma, allergic

rhinitis, nephritis, non-Hodgkin's lymphoma, noninsulin-dependent diabetes melitus (type II), non-small-cell lung cancer, organ transplantation, osteoarthritis, osteomalacia, osteopenia, osteoporosis, ovarian cancer, osteo-Behcet's disease, peptic ulcer, peripheral vascular disease, prostatic cancer, reflux esophagitis, renal failure, rheumatoid arthritis, schizophrenia, sepsis, septic shock, severe systemic fungal infection, small-cell lung cancer, spinal injury, stomach cancer, systemic lupus erythematosus, transient cerebral ischemic attack, pulmonary tuberculosis, valvular heart disease, vascular/multiple infarction-associated dementia, wound healing, insomnia, arthritis, and neurodegenerative disease, among other diseases. Therefore, in the technical field of the present invention, the novel physiologically active peptides having somatostatin-like or cortistatin-like activity and the large scale production method of the peptide are expected to enable development of novel drugs of value in the prevention or treatment of the above diseases.

[Problems to be solved by the Invention]

The present invention relates to novel peptides having useful physiological activities, precursors thereof, or salts thereof, DNAs coding for said peptides or precursors, recombinant vectors, transformants, a method of producing said peptides or precursors, pharmaceutical compositions containing said peptides or precursors, antibodies against said peptides or precursors, a method of screening and a kit for the screening of compounds and salts which are capable of modifying the binding of said peptides to receptors.

[Means for Solving the problems]

As a result of intensive investigations made by them for solving the above problems, the present inventors succeeded in cloning a cDNA having a novel base sequence by constructing primers based on the sequence information on an EST and carrying out RT-PCR using human brain poly(A)$^+$ RNA as the template. Further, the present inventors found that a useful somatostatin-like or cortistatin-like physiologically active peptide forms from the protein encoded by the cDNA obtained in the above manner. Based on these findings, the present inventors made further investigations. As a result, they have now completed the present invention.

The present invention thus provides:

(1) A peptide comprising the amino acid sequence defined under SEQ ID NO:1, a precursor thereof, or a salt of said peptide or precursor,

(2) A precursor mentioned in the above item (1) which comprises the amino acid sequence defined under SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6,

(3) A peptide or precursor mentioned in the above item (1) which has somatostatin-like or cortistatin-like activity,

(4) A DNA comprising a DNA having a nucleotide sequence coding for the peptide or precursor mentioned in the above item (1).

(5) A DNA mentioned in the above item (4) which comprises the nucleotide sequence defined under SEQ ID NO:7,

(6) A DNA mentioned in the above item (4) which comprises the nucleotide sequence defined under any of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12,

(7) A recombinant vector comprising the DNA mentioned in the above item (4),

(8) A transformant harboring the recombinant vector mentioned in the above item (7),

(9) A method of producing the peptide, precursor or salt mentioned in the above item (1) which comprises growing the transformant mentioned in the above item (8) to thereby cause production and accumulation of the peptide, precursor or salt mentioned in the above item (1) and harvesting the same,

(10) A pharmaceutical composition which comprises the peptide, precursor or salt mentioned in the above item (1),

(11) A pharmaceutical composition comprising the DNA mentioned in the above item (4),

(12) A pharmaceutical composition as mentioned in the above items (10) or (11) which is an agent for the treatment or prevention of hormone-producing tumors, acromegaly, giantism, dementia or, a hormone secretion inhibitor, a tumor growth inhibitor, or a neural activity or sleep modulator,

(13) An antibody against the peptide, precursor or salt mentioned in the above item (1),

(14) A method of screening for an antagonist or an agonist against a receptor for the peptide which comprise the peptide, precursor or salt as mentioned in the above item (1),

(15) A kit for screening for an antagonist or an agonist against a receptor for the peptide, which kit comprises the peptide, precursor or salt as mentioned in the above item (1).

The peptides having an amino acid sequence identical or substantially equivalent thereto identical to the amino acid sequence represented by SEQ ID NO:1 may be any of the peptides derived from various tissues of man and other warm-blooded animals (e.g. guinea pig, rat, mouse, fowl, rabbit, swine, sheep, bovine, monkey, etc.). Among such tissues are cells (e.g. hepatocytes, splenocytes, nerve cells, glia cells, β cells of pancreas, myelocytes, mesangial cells, Langerhans' cells, epidermic cells, epithelial cells, endothelial cells, fibroblasts, fibrocytes, myocites, adipocytes, immune cells (e.g. macrophages, T-cells, B cells, natural killer cells, mastocytes, neutrophils, basophils, eosinophils, monocytes), megarocytes, synovial cells, chondrocytes, osteocytes, osteoblasts, osteoclasts, mammary gland cells, hepatocytes, interstitial cells, the corresponding precursor cells, stem cells, cancer cells, etc.), all tissues in which such cells exist, for example the brain, various parts of the brain (e.g. olefactory bulb, amygdaloid body, basal ganglia, hippocampus, thalamus, hypothalamus, cerebral cortex, medula oblongata, cerebellum), spinal cord, pituitary gland, stomach, pancreas, kidney, liver, gonad, thyroid, gall-bladder, bone marrow, adrenal, skin, muscle, lung, bowels (e.g. large intestine and small intestine), blood vessel, heart, thymus, spleen, submandibular gland, peripheral blood, prostate, testis, ovary, placenta, uterus, bone, joint, skeletal muscle, etc. The peptides mentioned above may also be a synthetic peptides.

Examples of the amino acid sequence which is substantially equivalent to the amino acid sequence represented by SEQ ID NO:1.

Examples of the peptide of the present invention which comprises an amino acid sequence substantially equivalent to the amino acid sequence represented by SEQ ID NO:1 is a peptide having an amino acid sequence substantially equivalent to the amino acid sequence represented by SEQ ID NO:1, and having a qualitatively equivalent activity to the peptide having the amino acid sequence represented by SEQ ID NO:1.

The peptides of the present invention may be a mutein of the peptide comprising the amino acid sequence represented by SED ID NO:1.

The term "qualitatively equivalent activity" is used herein to mean substantial equivalence in qualitative terms such as a cortistatin-like or somatostatin-like activity, mentioned below. Therefore, the degree of equivalence may be different. However, differences in quantitative terms such as the potency of activity and the molecular mass of protein are immaterial.

And, the peptide of the present invention includes the peptides comprising an amino acid sequence wherein a few (1 to 5) amino acid residues are deleted from the amino acid sequence represented by SEQ ID NO:1, an amino acid sequence wherein a few (1 to 5) amino acid residues are substituted with the amino acid sequence represented by SEQ ID NO:1.

As typical examples of the deletion type mutein which are to be used, the following may be mentioned:

A peptide comprising an amino acid sequence (SEQ ID NO:2) derived from the amino acid sequence defined under SEQ ID NO:1 by deletion of two amino acids from the N terminus thereof etc.

The peptide of the present invention further includes those peptides in which the amino group of the N-terminal amino acid residue is protected by a protective group (e.g. C_{1-6} acyl, such as C_{1-6} alkanoyl, for example formyl, acetyl, etc.), those peptides having a pyroglutamyl group derived from a glutamyl group resulting from in vivo cleavage on the N-terminal side, those peptides in which one or more substituents (e.g. -OH, -SH, amino, imidazole group, indole group, guanidino) on the side chains of the intramolecular amino acids are protected with appropriate protective groups (e.g. C_{1-6} acyl such as C_{1-6} alkanoyl, for example formyl, acetyl; C_{1-6} alkyl such as methyl), complex peptides such as the so-called sugar peptides resulting from binding of a sugar chain, and the like.

The precursor of the present invention may be any peptide or protein provided that it contains the above-mentioned peptide of the present invention. For example, peptides or proteins resulting from addition of one or more amino acid residues to the N terminus or/and C terminus of the peptide of the invention are used. Among these, peptides or proteins resulting from addition of one or more amino acid residues to the N terminus of the peptide of the invention are preferred.

More specifically, those peptides resulting from addition of one or more amino acid residues (counted from the C terminus) of the amino acid sequence (composed of 88 amino acid residues) defined under SEQ ID NO:21 to the N terminus of the peptide having the amino acid sequence defined under SEQ ID NO:1, for example, are each used as the precursor of the present invention.

As examples of the precursor peptide comprising an amino acid sequence substantially the same as the amino acid sequence defined under SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6 which are to be used, the following may be mentioned:

(1) A peptide comprising an amino acid sequence derived from the amino acid sequence defined under SEQ ID NO:3 by deletion of about 1 to 10 amino acid residues therefrom, an amino acid sequence derived from the amino acid sequence defined under SEQ ID NO:3 by addition of about 1 to 15 amino acid residues thereto, or an amino acid sequence derived from the amino acid sequence defined under SEQ ID NO:3 by substitution of about 1 to 8 amino acid residues occurring therein by other amino acid residues;

(2) A peptide comprising an amino acid sequence derived from the amino acid sequence defined under SEQ ID NO:4 by deletion of about 1 to 15 amino acid residues therefrom, an amino acid sequence derived from the amino acid sequence defined under SEQ ID NO:4 by addition of about 1 to 10 amino acid residues thereto, or an amino acid sequence derived from the amino acid sequence defined under SEQ ID NO:4 by substitution of about 1 to 20 amino acid residues occurring therein by other amino acid residues;

(3) A peptide comprising an amino acid sequence derived from the amino acid sequence defined under SEQ ID NO:5 by deletion of about 1 to 10 amino acid residues therefrom, an amino acid sequence derived from the amino acid sequence defined under SEQ ID NO:5 by addition of about 1 to 10 amino acid residues thereto, or an amino acid sequence derived from the amino acid sequence defined under SEQ ID NO:5 by

substitution of about 1 to 20 amino acid residues occurring therein by other amino acid residues;

(4) A peptide comprising an amino acid sequence derived from the amino acid sequence defined under SEQ ID NO:6 by deletion of about 1 to 10 amino acid residues therefrom, an amino acid sequence derived from the amino acid sequence defined under SEQ ID NO:6 by addition of about 1 to 20 amino acid residues thereto, or an amino acid sequence derived from the amino acid sequence defined under SEQ ID NO:6 by substitution of about 1 to 20 amino acid residues occurring therein by other amino acid residues; and the like.

Like the above-mentioned peptide of the present invention, the precursor peptide of the invention further includes those peptides in which the amino group of the N-terminal amino acid residue is protected by a protective group, those peptides having a pyroglutamyl group derived from a glutamyl group resulting from in vivo cleavage on the N-terminal side, those peptides in which one or more substituents on the side chains of the intramolecular amino acids are protected with appropriate protective groups, and complex peptides such as the so-called sugar peptides resulting from binding of a sugar chain, and the like.

The peptides or precursors of this specification are represented in accordance with the conventions for description of peptides, that is the N-terminus (amino terminus) at left and the C-terminus (carboxyl terminus) at right. The peptide of the present invention including the protein containing the amino acid sequence of SEQ ID NO:1 is usually in the carboxyl (-COOH) or carboxylate

(-COO^-) form at the C-terminus but may be in the amide (-$CONH_2$) or ester (-COOR) form.

R in the ester residue includes a C_{1-6} alkyl group (e.g. methyl, ethyl, n-propyl, isopropyl, n-butyl, etc., a C_{3-8} cycloalkyl group (e.g. cyclopentyl, cyclohexyl, etc.), a C_{6-12} aryl group (e.g. phenyl, α-naphthyl, etc.), a C_{7-14} aralkyl group such as a phenyl-C_{1-2} alkyl group (e.g. benzyl, phenethyl, etc.) and α-naphthyl-C_{1-2} alkyl, (e.g. α-naphthylmethyl, etc.), as well as pivaloyloxymethyl group which is often used for the production of esters for oral administration.

When the peptides or precursor of the present invention has a carboxyl or a carboxylate function in any position other than the C-terminus, the corresponding carboxamide or ester form is also included in the scope of the present invention. The ester mentioned just above may be any of the esters mentioned for the C-terminal carboxyl group.

The salts of the peptide or the precursor of the present invention includes salts with physiologically acceptable acid addition salt. Examples of such salts are salts thereof with inorganic acids (e.g. hydrochloric acid, phosphoric acid, hydrobromic acid or sulfuric acid, etc.) and salts thereof with organic acids (e.g. acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid or benzenesulfonic acid, etc..)

The peptide, the precursor or a salt thereof of the present invention can be produced from the tissues or cells of human or other

warm-blooded animals by per se known purification technologies or, as described hereinafter, by culturing a transformant carrying a DNA encoding the protein. It can also be produced in accordance with the procedures for peptide synthesis which are described hereinafter.

When the peptide or the precursor of the present invention is produced from the tissues or cells of human or other warm-blooded animals, the tissues or cells of human or other warm-blood animals are homogenized and the peptide of the present invention is extracted by an acid, etc.. The peptide can be isolated and purified from the extracted solution by a combination of chromatography such as reverse phase chromatography, ion exchange chromatography and so on.

For the synthesis of the peptide, the precursor, or their salts, or their amide form of the present invention, any of commercial resins available for protein synthesis can be employed. Among such resins are chloromethyl resin, hydroxymethyl resin, benzhydrylamino resin, aminomethyl resin, 4-benzyloxybenzyl alcohol resin, 4-methylbenzhydrylamino resin, PAM resin, 4-hydroxymethyl-methylphenylacetamidomethyl resin, polyacrylamide resin, 4-(2',4'-dimethoxyphenyl-hydroxymethyl)phenoxy resin, and 4-(2',4'-dimethoxyphenyl-Fmoc-aminoethyl)phenoxy resin. Using such a resin, amino acids which may be beforehand protected at side-chain functional groups in a suitable manner can be serially condensed with the α-amino group in the order corresponding to the

amino acid sequence of the objective protein by various condensation techniques which are per se known. After completion of the final condensation reaction, the protein is separated from the resin and the protective groups are removed. Then, in highly diluted solution, the intramolecular disulfide-forming reaction is carried out to provide the objective proteins or amides thereof.

Referring to the above condensation of protected amino acids, various activating agents known to be useful for protein synthesis can be utilized, and carbodiimide reagents are especially preferred. The carbodiimide reagents include are DCC, N,N'-diisopropylcarbodiimide, and N-ethyl-N'-(3-dimethylaminoprolyl)carbodiimide and so on. For activation by these reagents, the protected amino acid and a racemization inhibitor (e.g. HOBt, HOOBt, etc.) can be directly added to the resin, or the protected amino acid can be activated beforehand in the form of symmetric acid anhydride, HOBt ester or HOOBt ester and, then, added to the resin.

The solvent used for the above-mentioned activation of protected amino acids or the conjugation thereof to the resin can be properly selected from among the solvents known to be useful for protein condensation reactions. Examples of the solvent are acid amides (e.g. N,N-dimethylformamide, N,N-dimethylacetamide, N-methylpyrrolidone, etc.), halogenated hydrocarbons (e.g. methylene chloride, chloroform, etc.), alcohols (e.g. trifluoroethanol, etc.), sulfoxides (e.g. dimethyl sulfoxide, etc.), ethers (e.g. pyridine, dioxane, tetrahydrofuran, etc.), nitriles

(e.g. acetonitrile, propionitrile, etc.), esters (e.g. methyl acetate, ethyl acetate, etc.), and suitable mixtures of these solvents. The reaction temperature can be selected from the range known to be useful for protein-forming reactions, usually the range of about -20℃ to about 50℃. The activated amino acid derivative is generally used in a 1.5 to 4-fold excess. When the condensation is found insufficient by ninhydrin assay, the reaction can be repeated to make the condensation thoroughly sufficient. When sufficient condensation can not be achieved by repeated reaction, an unreacted amino acid can be acetylated by using acetic anhydride or acetylimidazole so as not to effect a subsequent reaction.

The protective groups for protecting the amino group of the starting compound include Z, Boc, t-pentyloxycarbonyl, isobornyloxycarbonyl, 4-methoxy-benzyloxycarbonyl, Cl-Z, Br-Z, adamantyloxycarbonyl, trifluoroacetyl, phthaloyl, formyl, 2-nitrophenylsulfenyl, diphenylphosphinothioyl, Fmoc, and so on.

The carboxyl group can be protected in the form of, for example, an alkyl ester (e.g. straight-chain, branched, or cyclic alkyl esters such as methyl, ethyl, propyl, butyl, t-butyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, 2-adamantyl, and so on), an aralkyl ester (e.g. benzyl, 4-nitrobenzyl, 4-methoxybenzyl, 4-chlorobenzyl, benzhydryl, and so on), phenacyl ester, benzyloxycarbonylhydrazide, t-butoxycarbonylhydrazide or tritylhydrazide.

The hydroxyl group of serine can be protected in the form of an ester or an ether. The group suitable for esterification

includes carboxylic acid-derived acyl groups such as a lower(C_{1-6}) alkanoyl group (e.g. acetyl, etc.), an aroyl group (e.g. benzoyl, etc.), a benzyloxycarbonyl, an ethoxycarbonyl group and so on. The group suitable for etherification includes a benzyl group, a tetrahydropyranyl group, a t-butyl group and so on.

The protective group used for protecting the phenolic hydroxyl group of tyrosine includes Bzl, C^{12}-Bzl, 2-nitrobenzyl, Br-Z, t-butyl and so on.

The protective group for the imidazole group of histidine includes Tos, 4-methoxy-2,3,6-trimethylbenzenesulfonyl, DNP, benzyloxymethyl, Bum, Boc, Trt, Fmoc and so on.

The starting compound with activated carboxyl groups includes the corresponding acid anhydride, azide, and active ester (e.g. esters with alcohols such as pentachlorophenol, 2,4,5-trichlorophenol, 2,4-dinitrophenol, cyanomethyl alcohol, p-nitrophenol, HONB, N-hydroxysuccinimide, N-hydroxyphthalimide, HOBt, etc.). The starting compound with activated amino groups includes the corresponding phosphorylamide.

The method for removal of such protective groups includes catalytic reduction in a hydrogen stream in the presence of a catalyst (e.g. Pd black or Pd-on-carbon), acid treatment with anhydrous hydrogen fluoride, methanesulfonic acid, trifluoromethanesulfonic acid, trifluoroacetic acid or a mixture thereof, treatment with a base such as diiso-propylethylamine, triethylamine, piperidine, piperazine or the like, and reduction with sodium metal in liquid ammonia. The above deprotection by treatment with acid is generally

conducted at a temperature of about -20℃ to 40℃. This acid treatment can be carried out advantageously in the presence of a cation acceptor such as anisole, phenol, thioanisole, m-cresol, p-cresol, dimethyl sulfide, 1,4-butanedithiol, 1,2-ethanedithiol, or the like. The 2,4-dinitrophenyl group used for protecting the imidazole group of histidine can be removed by treatment with thiophenol, and the formyl group used for protecting the indole group of tryptophan can be removed not only by said acid treatment in the presence of 1,2-ethanedithiol, 1,4-butanedithiol or the like as described hereinbefore, but also by alkali treatment with diluted sodium hydroxide solution, diluted liquid ammonia, or the like.

The method for protecting any functional group that should not take part in the contemplated reaction, the protective group to be used for such protection, the method for eliminating the protective group, and the method for activating the functional group to be involved in the contemplated reaction can all be properly selected from among the known methods and groups.

An alternative method for providing the peptide or the precursor in amide form typically comprises protecting the α-carboxyl group of the C-terminal amino acid in the form of an amide, extending the peptide chain to a desired length towards the N-terminus, deprotecting the N-terminal α-amino acid of the resulting peptide chain selectively to provide an N-terminal-deprotected fragment, preparing a peptide fragment with its C-terminal carboxyl group selectively deprotected, and condensing the two fragments in a solvent such as the mixed solvent as mentioned above. The

condensation reaction can be carried out in the same manner as described hereinbefore. After purification of the protected peptide thus obtained by condensation, all the protective groups are eliminated by the procedures described hereinbefore to provide the contemplated peptide in a crude form. This crude peptide is purified by suitable known purification techniques and lyophilized to provide the desired peptide amide.

A method for providing the peptide or the precursor in an ester form comprises condensing the α -carboxyl group of the C-terminal amino acid with a suitable alcohol to prepare the corresponding ester and subjecting this ester to the same procedure as described for purification of the peptide amide to provide the objective peptide ester.

The precursor of the present invention is useful for producing the peptide of the present invention. Moreover, the precursor of the present invention has a substantially the same activity of the peptide of the present invention, that is cortistatin-like or somatostatin-like activity. Therefore, the precursor of the present invention has the same usefulness as of the peptide of the present invention.

Those peptide fragments, inclusive of salts thereof, which are formed on the occasion of production of the mature peptide of the present invention upon cleavage of the precursor of the invention are also physiologically useful peptides. Useful as such peptide fragments are, for example, peptides having the amino acid sequences defined under SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11

and SEQ ID NO:12, respectively, and the like. As the salts of these peptide fragments, there may be mentioned salts of the same kinds as those mentioned above regarding the salts of the peptide and precursor of the invention.

These peptide fragments and salts can be produced by cleaving the above-mentioned precursor of the invention using an appropriate peptidase, or according to the peptide synthesis technique to be mentioned later herein.

These peptide fragments and salts are also useful, for instance, as antigens for use in the production of antibodies against the precursor of the invention. These peptide fragments and salts are further important in elucidating the mechanisms of in vivo formation of the peptide of the invention. Furthermore, they have a central nervous system or reproductive function modulating effect and are useful as a central nervous system or reproductive function modulator as well.

The DNA coding for the peptide or precursor of the invention may be any DNA provided that it contains the nucleotide sequence coding for the above-mentioned peptide or precursor of the invention. It may be a genomic DNA, a genomic DNA library, a cDNA derived from the above-mentioned cells or tissue, a cDNA library derived from the above-mentioned cells or tissue, or a synthetic DNA. The vector to be used for library construction may be any of bacteriophages, plasmids, cosmids, phagemids and the like. Further, the total RNA or a mRNA fraction prepared from the above-mentioned cells or tissue may be used directly for amplification by the reverse transcriptase

polymerase chain reaction (hereinafter abbreviated as RT-PCR) technique.

Specifically, the DNA coding for a peptide having the same or substantially the same amino acid sequence as the amino acid sequence defined under SEQ ID NO:1 of the invention may, for example, be a DNA comprising the nucleotide sequence defined under SEQ ID NO:7 or any DNA having a nucleotide sequence capable of hybridizing with the nucleotide sequence defined under SEQ ID NO:7 and coding for a peptide having the same activities as those of the peptide having the amino acid sequence defined under SEQ ID NO:1 (e.g. somatostatin-like activity, cortistatin-like activity).

Useful as the DNA capable of hybridizing with the nucleotide sequence defined under SEQ ID NO:7 is, for example, a DNA comprising a nucleotide sequence having a homology of not less than about 70%, preferably not less than about 80%, more preferably not less than about 90% relative to the nucleotide sequence defined under SEQ ID NO:7.

More specifically, useful as the DNA coding for a peptide comprising the amino acid sequence defined under SEQ ID NO:1 is a DNA having the nucleotide sequence defined under SEQ ID NO:7, or the like.

The DNA coding for a deletion type mutein peptide of the present invention comprising the same or substantially the same amino acid sequence as the amino acid sequence defined under SEQ ID NO:3 is, for example, a DNA comprising the nucleotide sequence defined under SEQ ID NO:9 or any DNA having a nucleotide sequence capable of

hybridizing with the nucleotide sequence defined under SEQ ID NO:9 and coding for a peptide having the same activities as those of the peptide having the amino acid sequence defined under SEQ ID NO:3 (e.g. somatostatin-like activity, cortistatin-like activity).

Useful as the DNA capable of hybridizing is, for example, a DNA comprising a nucleotide sequence having a homology of not less than about 70%, preferably not less than about 80%, more preferably not less than about 90% relative to the nucleotide sequence defined under SEQ ID NO:9.

The DNA coding for a deletion type mutein peptide of the present invention comprising the same or substantially the same amino acid sequence as the amino acid sequence defined under SEQ ID NO:4 is, for example, a DNA comprising the nucleotide sequence defined under SEQ ID NO:10 or any DNA having a nucleotide sequence capable of hybridizing with the nucleotide sequence defined under SEQ ID NO:9 and coding for a peptide having the same activities as those of the peptide having the amino acid sequence defined under SEQ ID NO:4 (e.g. somatostatin-like activity, cortistatin-like activity).

Useful as the DNA capable of hybridizing is, for example, a DNA comprising a nucleotide sequence having a homology of not less than about 70%, preferably not less than about 80%, more preferably not less than about 90% relative to the nucleotide sequence defined under SEQ ID NO:10.

The DNA coding for a deletion type mutein peptide of the present invention comprising the same or substantially the same amino acid sequence as the amino acid sequence defined under SEQ ID NO:5 is,

for example, a DNA comprising the nucleotide sequence defined under SEQ ID NO:11 or any DNA having a nucleotide sequence capable of hybridizing with the nucleotide sequence defined under SEQ ID NO:11 and coding for a peptide having the same activities as those of the peptide having the amino acid sequence defined under SEQ ID NO:5 (e.g. somatostatin-like activity, cortistatin-like activity).

Useful as the DNA capable of hybridizing is, for example, a DNA comprising a nucleotide sequence having a homology of not less than about 70%, preferably not less than about 80%, more preferably not less than about 90% relative to the nucleotide sequence defined under SEQ ID NO:11.

The DNA coding for a deletion type mutein peptide of the present invention comprising the same or substantially the same amino acid sequence as the amino acid sequence defined under SEQ ID NO:6 is, for example, a DNA comprising the nucleotide sequence defined under SEQ ID NO:12 or any DNA having a nucleotide sequence capable of hybridizing with the nucleotide sequence defined under SEQ ID NO:12 and coding for a peptide having the same activities as those of the peptide having the amino acid sequence defined under SEQ ID NO:6 (e.g. somatostatin-like activity, cortistatin-like activity).

Useful as the DNA capable of hybridizing is, for example, a DNA comprising a nucleotide sequence having a homology of not less than about 70%, preferably not less than about 80%, more preferably not less than about 90% relative to the nucleotide sequence defined under SEQ ID NO:12.

The DNA coding for a peptide fragment formed on the occasion

of formation of a mature peptide from the above-mentioned precursor of the present invention may be any DNA provided that it contains a nucleotide sequence coding for the above-mentioned peptide fragment. It may be a genomic DNA, a genomic DNA library, a cDNA derived from the above-mentioned cells or tissue, a cDNA library derived from the above-mentioned cells or tissue, or a synthetic DNA.

For example, a DNA comprising the nucleotide sequence defined under SEQ ID NO:13 may be used as a DNA coding for a peptide fragment comprising the amino acid sequence defined under SEQ ID NO:17; a DNA comprising the nucleotide sequence defined under SEQ ID NO:14, for example, may be used as a DNA coding for a peptide fragment comprising the amino acid sequence defined under SEQ ID NO:18; a DNA comprising the nucleotide sequence defined under SEQ ID NO:15, for instance, may be used as a DNA coding for a peptide fragment comprising the amino acid sequence defined under SEQ ID NO:19; a DNA comprising the nucleotide sequence defined under SEQ ID NO:16, for instance, may be used as a DNA coding for a peptide fragment comprising the amino acid sequence defined under SEQ ID NO:20.

[Best Mode for Carrying Out the Present Invention]

As the means of cloning a DNA coding for the peptide or precursor of the present invention, there may be mentioned, for instance, (1) amplification of the desired DNA from the above-mentioned DNA library by the PCR technique using synthetic DNA primers having a partial nucleotide sequence of a DNA coding for the peptide or precursor of the present invention or (2) selection by hybridization of a DNA inserted into an appropriate vector with a labeled DNA fragment or

synthetic DNA coding for a part or the whole region of a peptide or precursor of the present invention.

The method of hybridization is the same as mentioned above. When a commercial library is used, it can be carried out following the method described in the use manual attached thereto.

Modification (deletion, addition, substitution) of DNA nucleotide sequences can be effected by a per se known method such as the gapped duplex method or Kunkel method, or a modification thereof, using a known kit, for example Mutan™-G (Takara Shuzo) or Mutan™-K (Takara Shuzo) or the like.

The thus-cloned DNA coding for a peptide or precursor of the present invention may be used as such or after restriction enzyme digestion or linker addition as desired, as the case may be. Said DNA has ATG as a translation initiation codon at the 5'-end thereof and may have TAA, TGA or TAG as a translation termination codon at the 3'-end thereof. It is also possible to add these translation initiation codon and translation termination codon using appropriate synthetic DNA adapters.

Expression vectors for the DNA coding for the peptide or precursor of the present invention can be produced, for example, by (a) excising the desired DNA fragment from a DNA coding for the peptide or precursor of the present invention and (b) joining said DNA fragment to an appropriate expression vector downstream of the promoter thereof.

The vector may include plasmids derived from Escherichia coli, e.g., pBR322, pBR325, pUC12, pUC13, etc.; plasmids derived from

Bacillus subtilis, e.g., pUB110, pTP5, pC194, etc.; plasmids derived from yeasts e.g., pSH19, pSH15, etc.; bacteriophages such as λ - phage: animal virus such as retrovirus, vaccinia virus, etc.; insect virus; and other vecters such as pA1-11, pXT1, pRc/CMV, pRc/RSV, pcDNAI/Neo and so on.

According to the present invention, any promoter can be used as long as it is appropriate for the host cell which is used for expressing a gene. When the host is an animal cell, the promoter include SRα, SV40 promoter, LTR promoter, CMV(cytomegalovirus) promoter, HSV-TK promoter, etc., and CMV promoter and SRα promoter are preferably used. When the host for the transformation is Escherichia coli, the promoter are preferably trp promoter, lac promoter, recA promoter, λPL promoter, lpp promoter, T7 promoter, etc.. When the host for the transformation is Bacillus, the promoter are preferably SPO1 promoter, SPO2 promoter, penP promoter, etc.. When the host is a yeast, the promoter are preferably PHO5 promoter, PGK promoter, GAP promoter, ADH promoter, AOX1 promoter, etc.. When the host is an insect cell, the promoter include polyhedrin promoter, P10 promoter, etc..

The expression vectors may, if necessary, further comprise enhancers, splicing signals, polyadenylation signals, selective markers, SV40 duplicate origin (hereinafter referred to as SV40 ori). Examples of selective markers are dihydrofolate reductase (hereinafter referred to as dhfr gene, ampicillin resistant gene (hereinafter referred to as Amp^r), neomycin-resistant gene (hereinafter referred to as Neo^r) and so on. The dhfr gene gives

methotrexate (MTX) registant and Neo gives G418 resistant. Particularly, when the dhfr gene is used as a selective marker against dhfr gene-deficient chinese hamster cell line, cells transfected by the objective gene can be selected in a thymidine-free medium.

Furthermore, an appropriate signal sequence for a host can be added to the N-terminal side of the protein. When the host is Escherichia coli, the utilizable signal sequences may include PhoA signal sequence, OmpA signal sequence, etc.. When the host is Bacillus, they may include α-amylase signal sequence, subtilisin signal sequence, etc.. When the host is a yeast, they may include MF signal sequence, SUC2 signal sequence, etc.. When the host is an animal cell, they may include insulin signal sequence, α-interferon signal sequence, antibody molecule signal sequence, etc..

A transformant or transfectant is obtained by using the vector thus constructed, which carries the DNA coding for the peptide of the present invention.

The host may be, for example, Escherichia species, Bacillus species, yeast cells, insect cells, insects, animal cells, etc..

Examples of Escherichia species include Escherichia coli K12.DH1 (Proceedings of the National Academy of Sciences of the United State of America, Vol. 60, 160 (1968)), JM103 (Nucleic Acids Research, Vol. 9, 309 (1981)), JA221 (Journal of Molecular Biology, Vol. 120, 517 (1978)), HB101 (Journal of molecular Biology, Vol, 41, 459 (1969)), C600 [Genetics, Vol. 39, 440 (1954)), etc..

Examples of Bacillus species are, for example, Bacillus subtilis MI114 (Gene, Vol. 24, 255 (1983)), 207-21 (Journal of

Biochemistry, Vol. 95, 87 (1984)), etc..

Examples of yeast cells are, for example, Saccharomyces cerevisiae AH22, AH22R$^-$, NA87-11A, DKD-5D or 20B-12, Schizosachcaromyces pombe NCYC1913 or Pichia pastoris KM71, etc..

Examples of insect cells are, for example, Spodoptera frugiperda cell (Sf cell), MG1 cell derived from a center intestine of Trichoplusia ni, High Five™ cell derived from eggs of Trichoplusia ni, Mamestra brassicae-derived cell, Estigmena acrea-derived cell and so on when virus is AcNPV; and Bombyx mori N cell (BmN cell) and so on when virus is BmNPV. Examples of the Sf cell are, for example, Sf9 cell (ATCC CRL 1711), Sf21 cell [both, Vaughn J.L. et al., In Vivo, 13, 213-217(1977)] and so on.

Examples of insects include a larva of silkworm (Bombyx mori larva) (Maeda et al., Nature, 315, 592(1985)).

Examples of animal cells are, for example, monkey-derived COS-7 cell line, Vero cell line, Chinese hamster ovary cell line (hereinafter referred to as CHO cell), dhfr gene-deficient Chinese hamster cell line (hereinafter referred to as CHO(dhfr$^-$) cell), mouse L cell, mouse AtT-20, mouse myeloma cell, rat GH3, humanFL, 293 cell, C127 cell, BALB3T3 cell, Sp-2/O cell, etc.. Among them, CHO cell, CHO(dhfr$^-$) cell, 293 cell, etc. are preferred.

Depending on host cells used, transformation is carried out using standard techniques appropriate to such cells.

Transformation of Escherichia species can be carried out in accordance with methods as disclosed in, for example, Proceedings of the National Academy of Sciences of the United State of America,

Vol. 69, 2110 (1972), and Gene, Vol. 17, 107 (1982), etc..

Transformation of Bacillus species can be carried out in accordance with methods as disclosed in, for example, Molecular & General Genetics, Vol. 168, 111 (1979), etc..

Transformation of yeast cells can be carried out in accordance with methods as disclosed in, for example, Methods in Enzymology, 194, 182-187(1991), etc..

Transformation of insect cells or insects can be carried out in accordance with methods as disclosed in, for example, Bio/Technology, 6, 47-55, (1988).

Transformation of animal cells can be carried out by methods as disclosed in, for example, Cell Engineering, separate vol. 8, New Cell Engineering Experiment Protocol, 263-267(1995) (Shujun Company), Virology, Vol. 52, 456 (1973), etc..

In introducing the expression vector into cells, known methods such as a calcium phosphate method (Graham, F. L. and van der Eb, A. J.: Virology, 52, 456-467(1973)), an electroporation (Neumann, E. et al., EMBO Journal, 1,841-845(1982)), etc. may be used.

In this way, a transformant transformed with the expression vector containing the DNA coding for the peptide or precursor of the present invention is obtained.

Meanwhile, as a method of allowing stable expression of the peptide or precursor of the present invention using animal cells, there may be mentioned the method comprising selecting, by clonal selection, animal cells in which the above expression vector introduced thereinto has been integrated into a chromosome. To be

concrete, transformant selection is carried out using the above-mentioned selective marker as an indicator. Further, the animal cells obtained in the above manner using the selective marker are subjected to repeated clonal selection, whereby a stable animal cell line capable of high level expression of the peptide or precursor of the present invention can be obtained. When the dhfr gene is used as the selective marker, it is also possible to obtain a higher expression animal cell line by culturing the cells while gradually raising the MTX concentration and selecting a resistant cell line and thereby intracellularly amplifying the DNA coding for the peptide or precursor of the present invention, together with the dhfr gene.

The peptide or precursor of the present invention, or a salt thereof, can be produced by culturing the transformant mentioned above under conditions enabling expression of the DNA coding for the peptide or precursor of the invention to thereby cause formation and accumulation of the peptide or precursor of the invention.

When a transformant the host of which is a strain of the genus Escherichia or Bacillus is cultured, a liquid medium is suited as the medium to be used in the cultivation, and carbon sources, nitrogen sources, inorganic and other materials necessary for the growth of said transformant are incorporated in said medium. As the carbon sources, there may be mentioned glucose, dextrin, soluble starch, sucrose and so forth. As the nitrogen sources, there may be mentioned inorganic or organic substances such as ammonium salts, nitric acid salts, corn steep liquor, peptone, casein, meat extract, soybean cake, and potato extract as well as inorganic materials such as calcium

chloride, sodium dihydrogen phosphate, magnesium chloride, etc. Yeast extract, vitamins, growth factors and the like may also be added. The pH of the medium is desirably about 5 to 8.

Preferred as the medium for culturing strains of the genus Escherichia is, for example, M9 medium containing glucose and casamino acids (Miller, Journal of Experiments in Molecular Genetics, 431-433, Cold Spring Harbor Laboratory, New York, 1972). When necessary, an agent such as 3β-indolylacrylic acid, for instance, may be added to said medium for efficient promoter functioning. When the host is a strain of the genus Escherichia, cultivation is carried out generally at about 15 to 43℃ for about 3 to 24 hours, if necessary with aeration and/or agitation.

When the host is a strain of the genus Bacillus, cultivation is carried out generally at about 30 to 40℃ for about 6 to 24 hours, if necessary with aeration and/or agitation.

As the medium for culturing a transformant where the host is a yeast, there may be mentioned, for example, Burkholder's minimum medium [Bostian, K. L. et al., Proc. Natl. Acad. Sci. USA, vol. 77, 4505 (1980)] and SD medium containing 0.5% casamino acids [Bitter, G. A. et al., Proc. Natl. Acad. Sci. USA, vol. 81, 5330 (1984)]. The pH of the medium is preferably adjusted to about 5 to 8. Cultivation is carried out generally at about 20℃ to 35℃ for about 24 to 72 hours, if necessary with aeration and/or agitation.

Useful as the medium for culturing a transformant where the host is an insect cell is Grace's insect medium [Grace, T. C. C., Nature, 195, 788 (1962)] supplemented with such additives as 10%

inactivated bovine serum in appropriate quantities. The pH of the medium is preferably adjusted to about 6.2 to 6.4. Cultivation is carried out generally at about 27℃ for about 3 to 5 days, if necessary with aeration and/or agitation.

Useful as the medium for culturing a transformant where the host is an animal cell are, for example, MEM medium containing about 5 to 20% fetal calf serum [Science, vol. 122, 501 (1952)], DMEM medium [Virology, vol. 8, 396 (1959)], RPMI 1640 medium [The Journal of the American Medical Association, vol. 199, 519 (1967)], 199 medium [Proceedings of the Society for the Biological Medicine, vol. 73, 1 (1950)] and the like. The pH is preferably about 6 to 8. Cultivation is carried out generally at about 30℃ to 40℃ for about 15 to 72 hours, if necessary with aeration and/or agitation.

Particularly when CHO (dhfr-) cells are used with the dhfr gene as a selective marker, the use of DMEM medium containing dialyzed fetal calf serum substantially free of thymidine is preferred.

The peptide or precursor of the present invention can be isolated and purified from the culture broth, for example in the following manner.

For extracting the peptide or precursor of the invention from cultured bacterial or other cells, an appropriate method can be used which comprises, for example, collecting bacterial or other cells after cultivation by a known method, suspending them in an appropriate buffer solution and disrupting them by means of supersonic waves, lysozyme and/or freezing-thawing, for instance, followed by centrifugation or filtration to give a crude extract containing the

peptide or precursor of the present invention. A protein denaturing agent such as urea or guanidine hydrochloride, and/or a surfactant such as Triton X-100™ may be contained in the buffer solution.

In cases where the peptide or precursor is excreted in the culture liquid phase, bacterial or other cells after completion of cultivation are separated from the supernatant by a per se known method and the supernatant is recovered. The peptide or precursor of the invention contained in the thus-obtained supernatant or extract can be purified by using per se known isolation/purification techniques in a suitable combination. As such known isolation/purification techniques, there may be mentioned techniques utilizing the difference in solubility, such as salting out or solvent precipitation, techniques principally utilizing the difference in molecular weight, such as dialysis, ultrafiltration, gel filtration and SDS-polyacrylamide gel electrophoresis, techniques utilizing the difference in electrostatic charge, such as ion exchange chromatography, techniques utilizing a specific affinity, such as affinity chromatography, techniques utilizing the difference in hydrophobicity, such as reversed-phase liquid chromatography, techniques utilizing the difference in isoelectric point, such as isoelectric focusing, and so on.

In cases where the peptide or the precursor of the present invention thus obtained is in a free form, the free-form peptide can be converted to a salt thereof by known methods or method analogous thereto. In case, where the peptide or the precursor thus obtained is in a salt form vice versa, the peptide salt can be converted to

a free form or to any other salt thereof by known methods or method analogous thereto.

The peptide or the precursor of the present invention produced by the transformant can be arbitrarily modified or a polypeptide can be partly removed therefrom, by a suitable protein-modifying enzyme before or after the purification. The protein-modifying enzyme may include trypsin, chymotrypsin, arginyl endopeptidase, protein kinase, glycosidase, etc.. The amount of the peptide or the precursor of the present invention thus obtained can be measured by an enzyme immunoassays (enzyme linked immunoassays) usingspecific antibodies.

The peptide, the precursor of the present invention or a salt thereof can be produced by per se known procedures for peptide synthesis. The peptide of the present invention can also be produced by cleaving the precursor of the present invention with a suitable peptidase. The process for peptide synthesis may be a solid-phase synthesis and/or a liquid-phase synthesis. Namely, the objective peptide can be produced by condensing a partial peptide or amino acid capable of constituting the protein with the residual part thereof and, when the product has a protective group, the protective group is removed whereupon a desire peptide can be manufactured. The known technology for condensation and deprotection includes the procedures described in the following literature (1)-(5).

(1) M. Bodanszky and M. A. Ondetti, Peptide Synthesis, Interscience Publishers, New York, 1966

(2) Schroeder and Luebke, The Peptide, Academic Press, New York,

1965

(3) Nobuo Izumiya et al., Fundamentals and Experiments in Peptide Synthesis, Maruzen, 1975

(4) Haruaki Yajima and Shumpei Sakakibara, Biochemical Experiment Series 1, Protein Chemistry IV, 205, 1977

(5) Haruaki Yajima (ed.), Development of Drugs-Continued, 14, Peptide Synthesis, Hirokawa Shoten

After the reaction, the peptide of the present invention can be isolated and purified by a combination of conventional purification techniques such as solvent extraction, distillation, column chromatography, liquid chromatography, and recrystallization. When the peptide isolated as above is in a free form, it can be converted to a suitable salt by known methods or method analogous thereto. On the other hand, when it is isolated as a salt, it can be converted to a free form or to any other salt thereof by known methods or method analogous thereto.

The antibodies against the peptide, the precursor of the present invention, or a salt thereof are any antibodies such as polyclonal antibodies and monoclonal antibodies which can recognize the peptide, the precursor of the present invention, or a salt thereof Among antibodies, the antibody which can neutralize the activity of the peptide, the precursor of the present invention, or a salt thereof is preferred.

The antibodies against the peptide, the precursor of the present invention, or a salt thereof (hereinafter, referred to as the peptide of the present invention) may be manufactured by methods

per se known to those of skill in the art or methods similar thereto, using the peptide of the present invention as antigen. For example, monoclonal antibodies and/or polyclonal antibodies can be manufactured by the method as given below.

Preparation of Monoclonal Antibody:

(a) Preparation of Monoclonal Antibody-Producing Cells

The peptide of the present invention is administered to warm-blooded animals either solely or together with carriers or diluents to the site favorable for antibody production. In order to potentiate the antibody productivity upon the administration, complete Freund's adjuvants or incomplete Freund's adjuvants may be administered. The administration is usually carried out once every 2 to 6 weeks and 2 to 10 times in total. Examples of the applicable warm-blooded animals are monkeys, rabbits, dogs, guinea pigs, mice, rats, sheep, goats and fowls. The use of mice and rats is preferred.

In establishing cells which produce monoclonal antibodies, an animal with the detectable antibody titer is selected from animals (e.g. mice) immunized with antigens, then spleen or lymph node is collected after 2 to 5 days from the final immunization and antibody-producing cells contained therein are fused with myeloma cells derived from homogeneous or heterogeneous animals to obtain monoclonal antibody-producing hybridomas. Measurement of the antibody titer in antisera may, for example, be carried out by reacting a labeled protein, which will be mentioned later, with the antiserum followed by measuring the binding activity of the labeling agent with the antibody. The cell fusion may be carried out, for example,

by a method of Koehler and Milstein (Nature, 256, 495, 1975). Examples of the fusion accelerator are polyethylene glycol (PEG), Sendai virus, etc. and the use of PEG is preferred.

Examples of the myeloma cells are those derived from warm-blooded animals such as NS-1, P3U1, SP2/0, AP-1, etc. and the use of P3U1 is preferred. The preferred fusion ratio of the numbers of antibody-producing cells used (spleen cells) to the numbers of myeloma cells is within a range of about 1:1 to 20:1. When PEG (preferably, PEG 1000 to PEG 6000) is added in a concentration of about 10 to 80% followed by incubating at 20 to 40℃, preferably, at 30 to 37℃, for 1 to 10 minutes, an efficient cell fusion can be carried out.

Various methods may be applied for screening a hybridoma which produces an anti-peptide etc. antibody. For example, a supernatant of hybridoma culture is added to a solid phase (e.g. microplate) to which the protein antigen is adsorbed either directly or with a carrier, then anti-immunoglobulin antibody (anti-mouse immunoglobulin antibody is used when the cells used for the cell fusion are those of mouse) which is labeled with a radioactive substance, an enzyme or the like, or protein A is added thereto and then anti-peptide etc. monoclonal antibodies bound on the solid phase are detected; or a supernatant of the hybridoma culture is added to the solid phase to which anti-immunoglobulin or protein A is adsorbed, then the protein labeled with a radioactive substance or an enzyme is added and anti-peptide etc. monoclonal antibodies bound with the solid phase is detected.

Selection and cloning of the anti-peptide etc. monoclonal antibody- producing hybridoma may be carried out by methods per se known to those of skill in the art or methods similar thereto. Usually, it is carried out in a medium for animal cells, containing HAT (hypoxanthine, aminopterin and thymidine). With respect to a medium for the selection, for the cloning and for the growth, any medium may be used so far as hybridoma is able to grow therein. Examples of the medium are an RPMI 1640 medium (Dainippon Pharmaceutical Co., Ltd., Japan) containing 1 to 20% (preferably 10 to 20%) of fetal calf serum (FCS), GIT medium (Wako Pure Chemical, Japan) containing 1 to 20% of fetal calf serum and a suitable serum-free medium for hybridoma (SFM-101; Nissui Seiyaku, Japan). The culture temperature is usually 20 to 40℃ and, preferably, about 37℃. The culture period is usually from five days to three weeks and, preferably, one to two weeks. The culture is usually carried out in 5% carbon dioxide gas. The antibody titer of the supernatant of the hybridoma culture may be measured by the same manner as in the above-mentioned measurement of the antibody titer in the antiserum.

(b) Purification of the Monoclonal Antibody

The separation and purification of the anti-peptide etc. monoclonal antibody may be carried out by methods for separating/purifying immunoglobulin such as salting-out, precipitation with alcohol, isoelectric precipitation, electrophoresis, adsorption/deadsorption using ion exchangers such as DEAE, ultracentrifugation, gel filtration, specific purifying methods in which only an antibody is collected by treatment with

an active adsorbent such as an antigen-binding solid phase, protein A or protein G and the bond is dissociated whereupon the antibody is obtained.

Preparation of Polyclonal Antibody:

The polyclonal antibody of the present invention can be produced by per se known methods or methods analogous thereto. The method comprises preparing an immunogen (antigen protein) per se or a conjugate of an imunogen with a carrier protein, immunizing a warm-blooded animal in the same manner as described for the production of the monoclonal antibody, harvesting a fraction containing the antibody against the peptide of the present invention from the immunized animal, and purifying the harvested antibody.

Referring to the immunogen-carrier protein conjugate for use in the immunization of a warm-blooded animal, the kind of carrier protein and the ratio of the carrier and hapten are not particularly restricted only if the production of the antibody against the hapten conjugated with the particular carrier protein and used for immunization proceeds efficiently. Thus, for example, bovine serum albumin, bovine thyroglobulin, hemocyanine, or the like is coupled in the weight ratio of about 0.1 to 20, preferably about 1 to about 5, to unity of the hapten.

A variety of condensing agents can be used for this coupling between the hapten and the carrier. Thus, for example, a glutaraldehyde, carbodiimide, maleimide, or a thiol or dithiopyridyl group-containing active ester reagent can be employed.

The condensation reaction product is administered to a

warm-blooded animal at a site favorable for antibody production, either as it is alone or together with a carrier or diluent. Enhancing antibody production, complete Freund's adjuvant or incomplete Freund's adjuvant may be administered. Administration is carried out generally once in about 2 to 6 weeks for a total of about 3 to 10 times.

The polyclonal antibody can be harvested from the blood, ascites fluid, or other body fluid, preferably from the blood, of the host warm-blooded animal.

The polyclonal antibody titer in the antiserum can be determined in the same manner as the determination of monoclonal antibody described hereinbefore. The separation and purification of the polyclonal antibody can be carried out by the same method as that described for the separation and purification of monoclonal antibody.

The antibody against the before-mentioned parial peptide which is preduced by processing of the precursor, can be produced and used, as mentioned-above. The DNA having a nucleotide sequence complementary or substantially complementary to the DNA coding for the protein, the precursor or the partial peptide of the present invention (hereinafter referred to as the DNA of the present invention) can be any DNA having a nucleotide sequence complementary or substantially complementary to that of the DNA of the present invention and capable of suppressing expression of the DNA.

The nucleotide sequence substantially complementary to the DNA of the present invention may, for example, be a nucleotide sequence

having an identity of not less than about 70%, preferably not less than about 80%, more preferably not less than about 90%, and for still better results, to the total nucleotide sequence or partial nucleotide sequence of the nucleotide sequence complementary to that the DNA of the present invention. Particularly preferred is an antisense DNA having an identity of not less than about 70%, preferably not less than about 80%, and more preferably not less than about 90%, and for still better results, not less than about 95% to the nucleotide sequence of the domain, of the complete nucleotide sequence complementary to that of the DNA of the present invention, which encodes the N-terminal region of the peptide of the present invention (e.g. the nucleotide sequence of the domain around the initiation codon). The antisense DNA can be synthesized using a known DNA synthesis hardware.

The peptide of the present invention, inclusive of a precursor thereof and a salt of said peptide or precursor, is a peptide having useful physiological activities such as somatostatin-like and/or cortistatin-like activity. More specifically, it has (1) growth hormone secretion inhibiting activity, (2) inhibitory activity against secretion of pituitary hormones such as thyroid stimulating hormone and prolactin, (3) inhibitory activity against secretion of gastrointestinal hormones such as gastrin and insulin, (4) neurotransmitter activity, (5) cell proliferation stimulating activity, (6) inhibitory activity against acetylcholine which is a REM sleep inducer, (7) smooth muscle contraction inhibiting activity, and so forth. Therefore, the peptide, precursor or salt

of the invention can be used in various applications.

In the following, several typical uses for the peptide, precursor or salt of the present invention (hereinafter sometimes referred to as the peptide or equivalent of the invention), the DNA coding for the peptide or precursor of the present invention (hereinafter sometimes referred to as the DNA of the invention), the antibody against the peptide, precursor or salt of the present invention (hereinafter sometimes referred to as the antibody of the invention) and the oligonucleotide derivative or a salt thereof are described.

(1) Drugs for the treatment or prevention of various diseases

As mentioned above, the peptide or equivalent of the invention has (1) growth hormone secretion inhibiting activity, (2) inhibitory activity against secretion of pituitary hormones such as thyroid stimulating hormone and prolactin, (3) inhibitory activity against secretion of gastrointestinal hormones such as gastrin and insulin, (4) neurotransmitter activity, (5) cell proliferation stimulating activity, (6) inhibitory activity against acetylcholine which is a REM sleep inducer, (7) smooth muscle contraction inhibiting activity and so on.

Therefore, the peptide or equivalent of the invention is useful as a drug for the treatment or prevention of various diseases, for example as hormone-producing tumors, acromegaly, giantism, dementia, diabetes, gastric ulcer or the like, a hormone secretion inhibitor, a tumor growth inhibitor, or a neural activity or sleep modulator. Furthermore, the peptide or equivalent of the invention or the DNA

of the invention is useful also as a drug, for example a therapeutic or prophylactic agent for various diseases such as acute bacterial meningitis, acute myocardial infarction, acute pancreatitis, acute viral encephalitis, adult respiratory distress syndrome, alcoholic hepatitis, Alzheimer's disease, asthma, arteriosclerosis, atopic dermatitis, bacterial pneumonia, bladder cancer, bone fracture, mammary cancer, hyperphagia, polyphagia, burn healing, carcinoma of the uterine cervix, chronic lymphatic leukemia, chronic myelocytic leukemia, chronic pancreatitis, hepatic cirrhosis, colorectal cancer (carcinoma of the colon/rectum), Crohn's disease, dementia, diabetic complications, e.g. diabetic nephropathy, diabetic neuropathy, diabetic retinopathy, etc., gastritis, Helicobacter pylori infection, hepatic insufficiency, hepatitis A, hepatitis B, hepatitis C, other types of heptatitis, herpes simplex virus infection, varicella-zoster virus infection, Hodgkin's disease, AIDS virus infection, human papilloma virus infection, hypercalcemia, hypercholesterolemia, hyperglyceridemia, hyperlipemia, infectious diseases, influenza virus infection, insulin-dependent diabetes melitus (type I), invasive staphylococcal infection, malignant melanoma, cancer metastasis, multiple myeloma, allergic rhinitis, nephritis, non-Hodgkin's lymphoma, noninsulin-dependent diabetes melitus (type II), non-small-cell lung cancer, organ transplantation, osteoarthritis, osteomalacia, osteopenia, osteoporosis, ovarian cancer, osteo-Behcet's disease, peptic ulcer, peripheral vascular disease, prostatic cancer, reflux esophagitis, renal failure, rheumatoid arthritis, schizophrenia, sepsis, septic shock, severe

systemic fungal infection, small-cell-lung cancer, spinal injury, stomach cancer, systemic lupus erythematosus, transient cerebral ischemic attack, pulmonary tuberculosis, valvular heart disease, vascular/multiple infarction-associated dementia, wound healing, insomnia, arthritis, and neurodegenerative disease, among other diseases. In particular, the peptide or equivalent of the invention or the DNA of the invention is useful as an agent for the treatment or prevention of insomnia.

Further, the DNA coding for the peptide or equivalent of the invention is useful as a drug for the treatment or prevention of various diseases, for example as hormone-producing tumors, acromegaly, giantism, dementia, diabetes, gastric ulcer or the like, a hormone secretion inhibitor, a tumor growth inhibitor, or a neural activity or sleep modulator. Furthermore, the DNA of the invention is useful also as a drug, for example a therapeutic or prophylactic agent for various diseases such as acute bacterial meningitis, acute myocardial infarction, acute pancreatitis, acute viral encephalitis, adult respiratory distress syndrome, alcoholic hepatitis, Alzheimer's disease, asthma, arteriosclerosis, atopic dermatitis, bacterial pneumonia, bladder cancer, bone fracture, mammary cancer, hyperphagia, polyphagia, burn healing, carcinoma of the uterine cervix, chronic lymphatic leukemia, chronic myelocytic leukemia, chronic pancreatitis, hepatic cirrhosis, colorectal cancer (carcinoma of the colon/rectum), Crohn's disease, dementia, diabetic complications, e.g. diabetic nephropathy, diabetic neuropathy, diabetic retinopathy, etc., gastritis, Helicobacter pylori

infection, hepatic insufficiency, hepatitis A, hepatitis B, hepatitis C, other types of heptatitis, herpes simplex virus infection, varicella-zoster virus infection, Hodgkin's disease, AIDS virus infection, human papilloma virus infection, hypercalcemia, hypercholesterolemia, hyperglyceridemia, hyperlipemia, infectious diseases, influenza virus infection, insulin-dependent diabetes melitus (type I), invasive staphylococcal infection, malignant melanoma, cancer metastasis, multiple myeloma, allergic rhinitis, nephritis, non-Hodgkin's lymphoma, noninsulin-dependent diabetes melitus (type II), non-small-cell lung cancer, organ transplantation, osteoarthritis, osteomalacia, osteopenia, osteoporosis, ovarian cancer, osteo-Behcet's disease, peptic ulcer, peripheral vascular disease, prostatic cancer, reflux esophagitis, renal failure, rheumatoid arthritis, schizophrenia, sepsis, septic shock, severe systemic fungal infection, small-cell-lung cancer, spinal injury, stomach cancer, systemic lupus erythematosus, transient cerebral ischemic attack, pulmonary tuberculosis, valvular heart disease, vascular/multiple infarction-associated dementia, wound healing, insomnia, arthritis, and neurodegenerative disease, among other diseases. In particular, the peptide or equivalent of the invention or the DNA of the invention is useful as an agent for the treatment or prevention of insomnia.

In the above-mentioned medical application of the peptide or equivalent of the invention, it can be administered orally in such dosage forms as optionally sugar-coated tablets, capsules, elixirs, microcapsules, etc., or parenterally in the form of an

injection which includes sterile solutions or suspensions in water or a pharmaceutically acceptable liquid medium other than water. Such dosage forms can be prepared, for example, by admixing the peptide or equivalent of the invention with one or more members of physiologically acceptable carriers, flavoring agents, excipients, vehicles, preservatives, stabilizers, binders and so forth according to the unit formulas generally required for pharmaceutical manufacture. The active ingredient contents of these preparations are such that an appropriate dose can be obtained within an indicated range.

Additives which can be mixed in tablets, capsules etc. include binders such as gelatin, corn starch, tragacanth and gum arabic, excipients such as crystalline cellulose, swelling agents such as corn starch, gelatin and alginic acid, lubricants such as magnesium stearate, sweetening agents such as sucrose, lactose and saccharin, and flavoring agents such as peppermint, akamono oil and cherry. When the unit dosage form is the capsule, the above-mentioned materials may further incorporate liquid carriers such as oils and fats. Sterile compositions for injection can be formulated by ordinary methods of pharmaceutical preparation such as by dissolving or suspending active ingredients, naturally occuring vegetable oils such as sesame oil and coconut oil, etc. in vehicles such as water for injection to create pharmaceutical compositions.

Aqueous liquids for injection include physiological saline and isotonic solutions containing glucose and other auxiliary agents, e.g., D-sorbitol, D-mannitol and sodium chloride, and may be used

in combination with appropriate dissolution aids such as alcohols, e.g., ethanol, polyalcohols, e.g., propylene glycol and polyethylene glycol, nonionic surfactants, e.g., polysorbate 80TM and HCO-50 etc. Oily liquids include sesame oil and soybean oil, and may be used in combination with dissolution aids such as benzyl benzoate and benzyl alcohol. Furthermore the above-mentioned materials may also be formulated with buffers, e.g., phosphate buffer and sodium acetate buffer; soothing agents, e.g., benzalkonium chloride, procaine hydrochloride; stabilizers, e.g., human serum albumin, polyethylene glycol; preservatives, e.g., benzyl alcohol, phenol; antioxidants etc. Normally, an appropriate ample is filled in with the thus-prepared pharmaceutical composition such as an injectable liquid.

The thus-obtained preparations are safe and of low toxicity and therefore can be administered, for example, to humans and warm-blooded animals (e.g. rat, mouse, guinea pig, rabbit, chicken, sheep, swine, cattle, horse, cat, dog, monkey, etc.).

The dose of the peptide or equivalent of the invention may vary depending on the disease to be treated, the subject of administration, the route of administration and other factors. Generally, however, a daily dose of about 0.1 to 100 mg, preferably about 1.0 to 50 mg, more preferably about 1.0 to 20 mg, of said peptide or equivalent is administered orally to human adults (whose body weight is assumed to be 60 kg), for the treatment of insomnia, for instance. In the case of parenteral administration, while the amount of the peptide or equivalent of the invention per dose may vary

depending on the subject of administration, the disease to be treated and other factors, the peptide or equivalent of the invention may conveniently be administered intravenously in the form of an injection to human adults (whose body weight is assumed to be 60 kg) for the treatment of insomnia, for instance, in a daily dose of about 0.01 to 30 mg, preferably about 0.1 to 20 mg, more preferably about 0.1 to 10 mg. In the case of other animal species, the dose corresponding to the above-mentioned dose for 60 kg body weight can be administered.

(2) Agent for genetic diagnosis

An abnormality in the DNA coding for the peptide or precursor of the invention (gene abnormality), if any, in human or other warm-blooded animals (e.g. rat, mouse, guinea pig, rabbit, chicken, sheep, swine, cattle, horse, cat, dog, monkey, etc.) can be detected by using the DNA of the invention as a probe. Therefore, said DNA is useful, for example as an agent for the genetic diagnosis of various diseases resulting from an impairment or mutation of the above-mentioned DNA or mRNA or decreased expression thereof or an increased level of said DNA or mRNA or excessive expression thereof.

Thus, the DNA of the invention is useful as an agent for genetic diagnosis of, for example, hormone-producing tumors, acromegaly, giantism, dementia; diabetes, gastric ulcer or the like, in addition, acute bacterial meningitis, acute myocardial infarction, acute pancreatitis, acute viral encephalitis, adult respiratory distress syndrome, alcoholic hepatitis, Alzheimer's disease, asthma, arteriosclerosis, atopic dermatitis, bacterial pneumonia, bladder

cancer, bone fracture, mammary cancer, hyperphagia, polyphagia, burn healing, carcinoma of the uterine cervix, chronic lymphatic leukemia, chronic myelocytic leukemia, chronic pancreatitis, hepatic cirrhosis, colorectal cancer (carcinoma of the colon/rectum), Crohn's disease, dementia, diabetic complications, e.g. diabetic nephropathy, diabetic neuropathy, diabetic retinopathy, etc., gastritis, Helicobacter pylori infection, hepatic insufficiency, hepatitis A, hepatitis B, hepatitis C, other types of heptatitis, herpes simplex virus infection, varicella-zoster virus infection, Hodgkin's disease, AIDS virus infection, human papilloma virus infection, hypercalcemia, hypercholesterolemia, hyperglyceridemia, hyperlipemia, infectious diseases, influenza virus infection, insulin-dependent diabetes melitus (type I), invasive staphylococcal infection, malignant melanoma, cancer metastasis, multiple myeloma, allergic rhinitis, nephritis, non-Hodgkin's lymphoma, noninsulin-dependent diabetes melitus (type II), non-small-cell lung cancer, organ transplantation, osteoarthritis, osteomalacia, osteopenia, osteoporosis, ovarian cancer, osteo-Behcet's disease, peptic ulcer, peripheral vascular disease, prostatic cancer, reflux esophagitis, renal failure, rheumatoid arthritis, schizophrenia, sepsis, septic shock, severe systemic fungal infection, small-cell-lung cancer, spinal injury, stomach cancer, systemic lupus erythematosus, transient cerebral ischemic attack, pulmonary tuberculosis, valvular heart disease, vascular/multiple infarction-associated dementia, wound healing, insomnia, arthritis, and neurodegenerative disease, among other

diseases. In particular, it is useful for diagnostic agent for insomnia.

(3) Assay of the peptide, precursor or salt of the invention

The antibody of the invention, which specifically recognizes the peptide or equivalent of the invention, can be used, for example, in assaying the peptide or equivalent of the invention in test solutions, in particular by the sandwich immunoassay technique.

Thus, the present invention provides:

(i) A method of assaying the peptide or equivalent of the invention in a test solution which comprises reacting an antibody against the peptide or equivalent of the invention competitively with the test solution and the peptide or equivalent of the invention which occurs in a labeled form and determining the proportion of the labeled peptide or equivalent of the invention that has been bound to said antibody; and

(ii) A method of assaying the peptide or equivalent of the invention in a test solution which comprises reacting the test solution with the antibody of the invention insolubilized on a carrier and another antibody of the invention which occurs in a labeled form either simultaneously or serially and determining the activity of the label on the insolubilizing carrier.

In the assay method mentioned above under (ii), it is desirable that one antibody be an antibody capable of recognizing the N-terminal sequence of the peptide or equivalent of the invention and the other antibody be an antibody capable of reacting with the C-terminal sequence of the peptide or equivalent of the invention.

Further, it is also possible to assay the peptide or equivalent of the invention using a monoclonal antibody against the peptide or equivalent of the invention (hereinafter referred to as monoclonal antibody of the invention) and, in addition, it is also possible to perform the detection by tissue staining, for instance. For achieving these objects, either the antibody molecule itself or a $F(ab')_2$, Fab' or Fab fraction of the antibody molecule may be used.

The method of assaying the peptide or equivalent of the invention using the antibody of the invention is not limited to any particular one but may be any assaying method that comprises detecting, by chemical or physical means, the amount of an antibody, antigen or antibody-antigen complex corresponding to the amount of the antigen (e.g. peptide amount) in a test solution and calculating the amount of said antigen using a standard curve constructed by using standard solutions containing known amounts of the antigen. Thus, for instance, the nephelometric, competitive, immunometric or sandwich technique may suitably be used. From the sensitivity and specificity viewpoint, the sandwich technique to be further mentioned later herein is particularly preferred.

As the label to be used in the assaying method using a labeled substance, there may be mentioned radioisotopes, enzymes, fluorescent substances and luminescent substances, among others. Preferred as the radioisotopes are, for example, [^{125}I], [^{131}I], [^{3}H], [^{14}C], etc. As the enzymes, those which are stable and high in specific activity are preferred and there may be mentioned, for example, β-galactosidase, β-glucosidase, alkaline phosphatase, peroxidase,

malate dehydrogenase, etc. As the fluorescent substances, there may be mentioned fluorescamine, fluoresceine isothiocyanate, etc. As the luminescent substances, there may be mentioned luminol, luminol derivatives, luciferin, lucigenin, etc. Further, the biotin-avidin system may also be used for antibody- or antigen-label coupling.

In insolubilizing the antigen or antibody, physical adsorption may be utilized, and chemical binding, which is generally used for insolubilization and fixation of peptides, enzymes or the like, may also be used. As the carrier, there may be mentioned insoluble polysaccharides such as agarose, dextran and cellulose, synthetic resins such as polystyrene, polyacrylamide and silicones, or glass and the like.

According to the sandwich technique, the peptide or equivalent of the invention in the test solution can be determined by reacting the test solution with the monoclonal antibody of the invention in an insolubilized form (first reaction) and further with another monoclonal antibody of the invention in a labeled form (second reaction) and measuring the activity of the label on the carrier used for insolubilization. The first and second reactions may be carried out in the reversed order. Further, they may be carried out simultaneously or one after the other. The label and the method of insolubilization may be the same as those mentioned hereinabove. Furthermore, in performing the immunoassay by the sandwich technique, it is not always necessary that only one antibody be used for the solid phase antibody or labeled antibody. A mixture of two or more

antibodies may be used for the purpose of improving the sensitivity of measurement, for instance.

In assaying the peptide or equivalent of the invention by the sandwich technique according to the invention, the monoclonal antibody to be used for the first reaction and the monoclonal antibody to be used for the second reaction are preferably antibodies differing in the site of binding to the peptide or equivalent of the invention. Thus, the antibodies to be used in the first and second reactions are such that when the antibody to be used for the second reaction recognizes a C-terminal portion of the peptide or equivalent of the invention, for instance, the antibody to be used for the first reaction should be an antibody recognizing a site other than the C-terminal portion, for example an N-terminal portion.

The monoclonal antibody of the invention can also be used in other measurement systems than the sandwich system, for example in competitive, immunometric or nephelometric systems.

The competitive technique comprises reacting the antigen in test solution and the labeled antigen competitively with the antibody, then separating the unreacted labeled antigen (F) from the labeled antigen (B) bound to the antibody (B/F separation), determining the amount of the label either on B or on F and thus assaying the antigen in the test solution. For this reaction mode, the liquid phase method using a soluble antibody as the antibody, polyethylene glycol for B/F separation, and a second antibody with respect to said antibody, among others, or the solid phase method using an insolubilized antibody as the first antibody or using a soluble first antibody,

and an immobilized second antibody may be employed.

According to the immunometric technique, the antigen in test solution and an immobilized antigen are competitively reacted with a predetermined amount of a labeled antibody and then the solid and liquid phases are separated from each other, or the antigen in test solution is reacted with an excessive amount of a labeled antibody, then an insolubilized antigen is added for causing the unreacted labeled antibody to be bound to the solid phase and, thereafter, the solid and liquid phases are separated from each other. Then, the amount of the label in either phase is determined and the amount of the antigen in test solution is calculated.

Further, in nephelometry, the amount of an insoluble precipitate resulting from the antigen-antibody reaction in a gel or solution is determined. Even when the amount of the antigen in test solution is small and gives the precipitate only in a minute amount, laser nephelometry, which utilizes scattering of laser beams, can be used with advantage.

In applying these respective immunological assay techniques to the assaying method of the invention, no particular conditions or operations are required. A system of assaying the peptide or equivalent of the invention may be constructed giving ordinary technical considerations, which are evident to those skilled in the art, to those conditions and procedures which are ordinary in the respective techniques. For details of these general technical means, reference may be made to several reviews, monographs and so on.

For instance, Hiroshi Irie (ed.): "Radioimmunoassay"

(published by Kodansha, 1974); Hiroshi Irie (ed.): "Radioimmunoassay, A Sequel" (published by Kodansha, 1979); Eiji Ishikawa et al. (ed.): "Koso Men-eki Sokuteiho (Enzyme Immunoassay)" (published by Igaku Shoin, 1978); Eiji Ishikawa et al. (ed.): "Koso Men-eki Sokuteiho", 2nd ed. (published by Igaku Shoin, 1982); Eiji Ishikawa et al. (ed.): "Koso Men-eki Sokuteiho", 3rd ed. (published by Igaku Shoin, 1987); Methods in Enzymology, vol. 70 (Immunochemical Techniques (Part A)), ibid., vol. 73 (Immunochemical Techniques (Part B)), ibid., vol. 74 (Immunochemical Techniques (Part C)), ibid., vol. 84 (Immunochemical Techniques (Part D: Selected Immunoassays)), ibid., vol. 92 (Immunochemical Techniques (Part E: Monoclonal Antibodies and General Immunoassay Methods)), ibid., vol. 121 (Immunochemical Techniques (Part I: Hybridoma Technology and Monoclonal Antibodies)) (published by Academic Press) and so on may be referred to.

In the above manner, the peptide or equivalent of the invention can be assayed with good sensitivity by using the antibody of the invention.

Furthermore, various diseases in which the peptide or equivalent of the invention is involved can be diagnosed by determining the concentration of the peptide or equivalent of the invention using the antibody of the invention.

More specifically, when a reduced concentration of the peptide or equivalent of the invention is detected, the diagnosis may be such that the disease suspected is a hormone-producing tumor, acromegaly, giantism, dementia, diabetes, gastric ulcer, or insomnia, for instance, or that the possibility of manifestation of such disease

in the future is high.

When, on the other hand, an increased concentration of the peptide or equivalent of the invention is detected, the diaganosis may be such that the disease suspected is dwarfism, agalactia/hypogalactia, or diabetes, for instance, or that the possibility of manifestation of such disease in the future is high.

In addition, when an abnormal concentration of the peptide of the invention is detected, the diagnosis may be such that the disease suspected is acute bacterial meningitis, acute myocardial infarction, acute pancreatitis, acute viral encephalitis, adult respiratory distress syndrome, alcoholic hepatitis, Alzheimer's disease, asthma, arteriosclerosis, atopic dermatitis, bacterial pneumonia, bladder cancer, bone fracture, mammary cancer, hyperphagia, polyphagia, burn healing, carcinoma of the uterine cervix, chronic lymphatic leukemia, chronic myelocytic leukemia, chronic pancreatitis, hepatic cirrhosis, colorectal cancer (carcinoma of the colon/rectum), Crohn's disease, diabetic complications, e.g. diabetic nephropathy, diabetic neuropathy, diabetic retinopathy, etc., gastritis, Helicobacter pylori infection, hepatic insufficiency, hepatitis A, hepatitis B, hepatitis C, other types of heptatitis, herpes simplex virus infection, varicella-zoster virus infection, Hodgkin's disease, AIDS virus infection, human papilloma virus infection, hypercalcemia, hypercholesterolemia, hyperglyceridemia, hyperlipemia, miscellaneous infectious diseases, influenza virus infection, insulin-dependent diabetes melitus (type I), invasive

staphylococcal infection, malignant melanoma, cancer metastasis, multiple myeloma, allergic rhinitis, nephritis, non-Hodgkin's lymphoma, noninsulin-dependent diabetes melitus (type II), non-small-cell lung cancer, organ transplantation, osteoarthritis, osteomalacia, osteopenia, osteoporosis, ovarian cancer, osteo-Behcet's disease, peptic ulcer, peripheral vascular disease, prostatic cancer, reflux esophagitis, renal failure, rheumatoid arthritis, schizophrenia, sepsis, septic shock, severe systemic fungal infection, small-cell lung cancer, spinal injury, stomach cancer, systemic lupus erythematosus, transient cerebral ischemic attack, pulmonary tuberculosis, valvular heart disease, vascular/multiple infarction-associated dementia, wound healing, arthritis, and neurodegenerative disease, among other diseases, or that the possibility of manifestation of such disease in the future is high.

(4) Screening for candidate medicinal compounds

The peptide or equivalent of the invention is specifically conjugated with somatostatin receptors, receptors for the peptide or equivalent of the invention, and those receptors, such as GPR7 and GPR8, to which the peptide or equivalent of the invention may be conjugated (hereinafter collectively referred to as "receptor(s)" for short) and, therefore, by constructing a ligand-receptor binding assay system using the peptide or equivalent of the invention and said receptor, it is possible to carry out screening for candidate medicinal compounds having somatostatin-like or cortistatin-like activity, or screening for candidate medicinal compounds capable

of stimulating or inhibiting the activity of the peptide or equivalent of the invention or of somatostatin or cortistatin. Thus, the present invention provides a method of screening for a compound, or a salt thereof, which is capable of modifying the binding of the peptide or equivalent of the invention to said receptor or receptors which comprises using the peptide or equivalent of the invention.

More specifically, the present invention provides:

(I) A method of screening for a compound, or a salt thereof, which is capable of modifying the binding of the peptide or equivalent of the invention to the receptor, which comprises, on the one hand, (i) bringing the peptide or equivalent of the invention into contact with said receptor, a fragment peptide derived therefrom, or a salt of said receptor or fragment peptide and, on the other hand, (ii) bringing the peptide or equivalent of the invention and a compound to be tested into contact with said receptor, fragment peptide or salt, and making a comparison between the above cases (i) and (ii); and

(II) A method of screening for a compound, or a salt thereof, which is capable of modifying the binding of the peptide or equivalent of the invention to the receptor, which comprises, on the one hand, (i) bringing the peptide or equivalent of the invention into contact with cells or a cell membrane fraction, which contain or contains said receptor and, on the other hand, (ii) bringing the peptide or equivalent of the invention and a compound to be tested into contact with the cells or cell membrane fraction containing said receptor, and making a comparison between the above cases (i) and (ii).

More specifically, the screening method of the invention is characterized in that the levels of binding of the peptide or equivalent of the invention to said receptor or receptor-containing cells, or the cell stimulating activities, for instance, are determined or measured in the cases (i) and (ii) and compared therebetween.

More specifically, the present invention provides:

(1a) A method of screening for a compound, or a salt thereof, which is capable of modifying the binding of a peptide or equivalent of the invention to the receptor, which comprises, on the one hand, (i) bringing the peptide or equivalent of the invention in a labeled form into contact with said receptor, a fragment peptide derived therefrom or a salt of said receptor or fragment peptide and, on the other hand, (ii) bringing the labeled peptide or equivalent of the invention and a compound to be tested into contact with said receptor, fragment peptide or salt, and determining and comparing the levels of binding of the labeled peptide or equivalent of the invention to said receptor, fragment peptide or salt in and between the above cases (i) and (ii);

(2a) A method of screening for a compound, or a salt thereof, which is capable of modifying the binding of the peptide or equivalent of the invention to the receptor, which comprises, on the one hand, (i) bringing the peptide or equivalent of the invention in a labeled form into contact with cells or a cell membrane fraction, which contain or contains said receptor and, on the other hand, (ii) bringing the labeled peptide or equivalent of the invention and a compound to

be tested into contact with said receptor-containing cells or cell membrane fraction, and determining and comparing the levels of binding of the labeled peptide or equivalent to said cells or cell membrane fraction in and between the above cases (i) and (ii);

(2b) A method of screening for a receptor agonist which comprises bringing the peptide or equivalent of the invention into contact with cells containing the receptor and determining and comparing the thus-obtained data on cell stimulating activities mediated by said receptor (e.g. arachidonic acid release, acetylcholine release, intracellular Ca^{2+} concentration change, intracellular cAMP formation, intracellular cGMP formation, inositol phosphate production, cell membrane potential change, intracellular protein phosphorylation, c-fos activation, pH decrease, cell migration activity promoting or inhibiting activity, etc., in particular intracellular cAMP formation promoting or inhibiting activity); and

In the above-mentioned screening method (1a) or (2a), compounds capable of binding to the receptor and modifying (or inhibiting) the binding of the peptide or equivalent of the invention to the receptor can be selected as receptor agonists or receptor antagonists.

In the above-mentioned screening method (1a) or (2a), compounds incapable of binding to the receptor but capable of increasing the binding of the peptide or equivalent of the invention to the receptor can be selected as compounds capable of increasing the binding of the peptide or equivalent of the invention to the receptor.

In the above-mentioned screening method (1a) or (2a), compounds incapable of binding to the receptor but decreasing the binding of the peptide or equivalent of the invention to the receptor can be selected as compounds capable of decreasing the binding of the peptide or equivalent of the invention to the receptor.

In the above-mentioned screening method (2b), compounds capable of binding to the receptor and inhibiting the receptor-mediated cell stimulating activity (e.g. arachidonic acid release, acetylcholine release, intracellular Ca^{2+} concentration change, intracellular cAMP formation, intracellular cGMP formation, inositol phosphate production, cell membrane potential change, intracellular protein phosphorylation, c-fos activation, pH decrease, cell migration activity promoting or inhibiting activity, etc.), in particular inhibiting intracellular cAMP formation can be selected as receptor agonists.

Among the receptors to be used in the screening method of the invention, the somatostatin receptor includes, among others, somatostatin receptor subtype 1 (SSTR1) or subtype 2 (SSTR2) (Yamada et al., Proc. Natl. Acad. Sci., USA, vol. 89, pp. 251-255, 1992), subtype 3 (SSTR3) (Yamada et al., Molecular Endocrinology, vol. 6, pp. 2136-2142, 1992), subtype 4 (SSTR4) or subtype 5 (SSTR5) (Yamada et al., Biochem. Biophys, Res. Commun., vol. 195, pp. 844-852, 1993), etc. As GPR7 or GPR8, those described in Genomics, 28, 84-91 (1995) can be used. The receptor for the peptide or equivalent of the invention can be obtained by per se known techniques for protein purification and it is also possible to obtain the desired receptor

by cloning a DNA coding for said receptor by per se known genetic engineering techniques and then causing the expression of said DNA according to the above-mentioned method of causing expression of the peptide or equivalent of the invention.

Usable as the receptor-derived fragment peptide are fragment peptides obtained by appropriate cleavage of the full-length peptide.

Usable as the receptor-containing cells are such cells as those mentioned above as the host cells for use in the expression of the peptide or equivalent of the invention. Among them, CHO cells or the like are preferred, however. The receptor-containing cells can be produced by using a DNA coding for the receptor and according to per se known techniques, for example the above-mentioned method for the expression of the peptide of the invention. The DNA coding for the receptor can be obtained by per se known genetic engineering techniques, and somatostatin receptor subtypes 1 to 5 and GPR7 or GPR 8, for instance, can be obtained according to the references cited above.

When said receptor-containing cells are used in the screening method of the invention, said cells may be fixed with glutaraldehyde, formalin or the like. The fixation can be carried out according to per se known techniques. Further, brain, hypophysis, lung and other tissues derived from various animals and membrane fractions thereof may be used as the receptor-containing cells.

The labeled peptide or equivalent of the invention is, for example, the peptide or equivalent of the invention labeled with [^{3}H], [^{125}I], [^{14}C] or [^{35}S], or the like.

As the test compound, there may be mentioned peptides, proteins, non-peptide compounds, synthetic compounds, fermentation products, cell extracts, plant extracts, animal tissue extracts and so forth. These compounds may be novel ones or known ones.

Specifically, in carrying out the above-mentioned screening method (Ia) or (IIa), receptor standards are first prepared by suspending cells or a cell fraction, which contain or contains the receptor of the invention or the receptor or a fragment peptide thereof in a buffer suited for screening. The buffer may be any buffer that will not inhibit the binding of the peptide or equivalent of the invention to the receptor, for example phosphate buffer, Tris-hydrochloride buffer or the like, which has a pH of about 4 to 10 (desirably about 6 to 8). For reducing non-specific binding, a surfactant, such as CHAPS, Tween-80™ (Kao-Atlas), digitonin or deoxycholate, may also be added to the buffer. For inhibiting receptor or ligand decomposition by proteases, a protease inhibitor, such as PMSF, leupeptin, bacitracin, aprotinin, E-64 (product of Peptide Institute) or pepstatin, may further be added. When, on the other hand, the cells are fixed or immobilized ones, the binding of the peptide or equivalent of the invention to the receptor may be effected by using the cells in a state immobilized on incubation vessels, namely in the form of cells as grown, or cells fixed with glutaraldehyde or paraformaldehyde.

In this case, a culture medium or Hank's solution, among others, is used as said buffer. And a predetermined amount (e.g. about 10,000 cpm to 1,000,000 cpm in the case of 2,000 Ci/mmol) of the peptide

or equivalent of the invention in a labeled form (e.g. [^{125}I]-labeled peptide or equivalent of the invention) is added to 0.01 ml to 10 ml of the receptor solution and, at the same time, 10^{-4} M to 10^{-10} M of the test compound is caused to coexist. To ascertain the non-specific binding (NSB), reaction tubes with a large excess of the peptide or equivalent of the invention added in an unlabeled form are also prepared. The reaction is carried out at about 0℃ to 50℃, desirably about 4℃ to 37℃, for about 20 minutes to 24 hours, desirably about 30 minutes to 3 hours. After the reaction, each reaction mixture is filtered through a glass fiber filter or the like and, after washing with an appropriate amount of the same buffer, the radioactivity (e.g. radioactivity of [^{125}I]) remaining on the glass fiber filter is measured using a liquid scintillation counter or γ-counter. For the filtration, a manifold or cell harvester may be used; the use of a cell harvester is desirable for improving the efficiency, however. When the count (B_0) in the absence of any antagonizing substance minus the non-specific binding (NSB), namely the count (B_0 - NSB), is taken as 100%, a test compound showing a specific binding (B - NSB) which is not more than 50%, for instance, of the count (B_0 - NSB) can be selected as a candidate agonist or antagonist.

In carrying out the above-mentioned screening method (IIb), the receptor-mediated cell stimulating activity (e.g. arachidonic acid release, acetylcholine release, intracellular Ca^{2+} concentration change, intracellular cAMP formation, intracellular cGMP formation, inositol phosphate production, cell membrane potential

change, intracellular protein phosphorylation, c-fos activation, pH decrease, cell migration activity promoting or inhibiting activity, etc.) can be measured using a known method or a commercial assay kit. Specifically, cells containing the receptor are first cultured on multiwell plates or the like. Prior to carrying out the screening, the medium is exchanged for a fresh medium or an approrpiate buffer showing no cytotoxicity. The test compound etc. are then added and, after a predetermined incubation period, the cells are extracted or the supernatant is recovered, and the product or products formed are assayed by the respective methods. If the detection of formation of a substance (e.g. arachidonic acid) selected as the indicator of cell stimulating activity is confounded by a decomposing enzyme present in the cells, the assay may be carried out in the presence of an inhibitor of said decomposing enzyme. As regards cAMP production inhibiting activity or the like, the activity can be detected in terms of the inhibitory activity against cells in which the basal production has been augmented with forskolin or the like.

The screening kit of the invention comprises the peptide or equivalent, preferably together with cells or a cell membrane fraction which contain or contains the receptor or the receptor or a fragment peptide thereof.

As examples of the screening kit of the invention, there may be mentioned the following:

[Reagents for screening]

① Measurement buffer and washing buffer

Hank's balanced salt solution (Gibco) supplemented with 0.05%

bovine serum albumin (Sigma).

This is sterilized by filtration through a filter with a pore size of 0.45 μm and stored at 4℃. It may be prepared extemporaneously.

② Somatostatin receptor standard

Somatostatin receptor-containing CHO cells subcultured on 12-well plates at 5×10^5 cells/well and cultured under the conditions of 37℃ and 5% CO_2 plus 95% air for 2 days.

③ Labeled peptide or equivalent of the invention

The peptide or equivalent of the invention as labeled with commercially available [^{3}H], [^{125}I], [^{14}C], [^{35}S] or the like (e.g. [^{125}I]hCS-17).

It is stored in a solution state at 4℃ or -20℃ and extemporaneously diluted to 1 μM with measurement buffer.

④ Standard solution of the peptide or equivalent of the invention

The peptide or equivalent of the invention is dissolved in PBS containing 0.1% bovine serum albumin (Sigma) to 0.1 mM and stored at -20℃.

[Method of measurement]

① Recombinant somatostatin receptor-containing CHO cells cultured on 12-well tissue culture plates are washed with two 1-ml portions of measurement buffer, and 490 μl of measurement buffer is added to each well.

② 5 μl of a 10^{-3} to 10^{-10} M solution of the test compound is added, then 5 μl of a 5 nM solution of the peptide or equivalent of the invention in a labeled form is added, and the reaction is allowed

to proceed at room temperature for 1 hour. To ascertain the non-specific binding, 5 μl of a 10^{-4} M solution of the peptide or equivalent of the invention is added in lieu of the test compound.

③ The reaction solution is removed, and each well is washed with three 1-ml portions of washing buffer. The cell-bound labeled peptide or equivalent of the invention is dissolved using 0.5 ml of 0.2 N NaOH-1% SDS and the solution is mixed with 4 ml of a liquid scintillator A (Wako Pure Chemical Industries).

④ The radioactivity is measured using a liquid scintillation counter (Beckman) and expressed in terms of percent maximum binding (PMB) according to the formula [Mathematical Formula 1] shown below. When the label is [^{125}I], the radioactivity can be measured directly using a gamma counter without admixing with the liquid scitillator.

[Mathematical Formula 1]

$$PBM = [(B - NSB)/(B0 - NSB)] \times 100$$

where PMB: percent maximum binding;

B : value when the test compound is added;

NSB : non-specific binding;

B_0 : maximum binding.

As mentioned above, the peptide or equivalent of the invention is useful as a reagent for screening for a receptor-agonist or a receptor-antagonist.

The compound, inclusive of salts thereof, obtained by using the screening method or screening kit of the invention is a compound capable of modifying the binding of the peptide or equivalent of the invention to the receptor and, more particularly, a compound

capable of binding to the receptor to inhibit stimulation of cells by agonists (the so-called receptor agonist), a compound capable of binding to the receptor and inhibiting a cell stimulating activity (the so-called receptor antagonist), a compound capable of increasing the binding of the peptide or equivalent of the invention to the receptor or a compound capable of diminishing the binding of the peptide or equivalent of the invention to the receptor.

The receptor agonist has all or some of the physiological activities of the peptide or equivalent of the invention or somatostatin, hence is useful as a drug, which is safe and low in toxicity, depending on its physiological activities. For instance, it is useful as an inhibitor of the secretion of such hormones as growth hormone, pituitary hormones (e.g. thyroid stimulating hormone, prolactin, etc.) gastrointestinal hormones (e.g. gastrin, insulin, etc.), etc. Furthermore, it is useful as a therapeutic or prophylactic agent for hormone-producing tumors, acromegaly, gigantism, dementia, diabetes, gastric ulcer and other diseases, or as a hormone secretion inhibitor, a tumor growth inhibitor, a neural activity or sleep modulator, or the like.

On the other hand, the receptor antagonist inhibits all or some of the physiological activities of the peptide or equivalent of the invention or somatostatin, hence is useful as a safe and low-toxicity drug for inhibiting such physiological activities. For instance, it is useful as an promoter of the secretion of such hormones as growth hormone, pituitary hormones (e.g. thyroid stimulating hormone, prolactin, etc.), gastrointestinal hormones (e.g. gastrin,

insulin, etc.) or the like. It is further useful as a therapeutic or prophylactic agent for dwarfism, agalactia/hypogalactia, diabetes, etc., or a modulator of the functions of digestion-related organs (e.g. function modulator for such organs as stomach, small intestine, pancreas, liver, etc.).

The compound capable of increasing the binding of the peptide or equivalent of the invention to the receptor enhances the physiological activities of the peptide or equivalent of the invention or somatostatin, hence is useful as a drug of the same nature as the above-mentioned receptor agonist.

The compound capable of diminishing the binding of the peptide or equivalent of the invention to the receptor suppresses the physiological activities of the peptide or equivalent of the invention or somatostatin, hence is useful as a drug of the same nature as the above-mentioned receptor antagonist.

In using it as the above-mentioned therapeutic or prophylactic agent, the compound obtained by using the screening method or screening kit of the invention can be used in a conventional manner. For instance, it can be made up into pharmaceutical preparations or dosage forms, such as tablets, capsules, elixirs, microcapsules, sterile solutions or suspensions, in the same manner as in the case of the above-mentioned drug composition containing the peptide or equivalent of the invention, and can be administered to human or warm-blooded animals.

The thus-obtained preparations are safe and low in toxicity; hence can be administered to human or warm-blooded animals (e.g.

mouse, rat, rabbit, sheep, swine, cattle, horse, chicken, cat, dog, monkey, chimpanzee, etc.), for instance.

The dose of said compound may vary depending on the disease to be treated, the subject of administration, the route of administration and other factors. Generally, however, where the receptor agonist is orally administered for the treatment of insomnia, for instance, said receptor agonist is administered in a daily dose of about 0.1 mg to 100 mg, preferably about 1.0 to 50 mg, more preferably about 1.0 to 20 mg per adult human (assuming that the body weight is 60 kg). For nonoral administration, the unit dose of said receptor agonist also may vary depending on the subject of administration, the disease to be treated and other factors but, in the case of administration of said receptor agonist in the form of an injection to an average adult (weighing 60 kg) for the treatment of insomnia, for instance, it is advisable that said receptor agonist be administered by intravenous injection in a daily dose of about 0.01 to 30 mg, preferably about 0.1 to 20 mg, more preferably about 0.1 to 10 mg. In the case of other animals, a dose corresponding to the above-mentioned 60 kg-base dose can be administered.

On the other hand, for oral administration of the receptor antagonist for the treatment of dwarfism, said receptor antagonist is generally administered to human adults (weighing 60 kg) in a daily dose of about 0.1 mg to 100 mg, preferably about 1.0 to 50 mg, more preferably about 1.0 to 20 mg. In the case of nonoral administration, the unit dose of said receptor antagonist also may vary depending on the subject of administration, the disease to be treated and other

factors. In parenteral administration, in the form of an injection, to an ordinary adult (weighing 60 kg) for the treatment of dwarfism, it is advisable that said receptor antagonist be administered by intravenous injection in a daily dose of about 0.01 to 30 mg, preferably about 0.1 to 20 mg, more preferably about 0.1 to 10 mg. In other animals, too, a dose corresponding to the above-mentioned 60 kg-base dose can be administered.

(4) Antisense DNA

As mentioned hereinbefore, the peptide or equivalent of the invention has (i) growth hormone secretion inhibiting activity, (ii) inhibitory activity against secretion of pituitary hormones such as thyroid stimulating hormone and prolactin, (iii) inhibitory activity against secretion of gastrointestinal hormones such as gastrin and insulin, (iv) neurotransmitter activity, (v) cell proliferation stimulating activity, (vi) inhibitory activity against activities of acetylcholine, which is a REM sleep inducer, and (vii) smooth muscle contraction inhibiting activity, among others.

Therefore, the oligonucleotide derivative A promotes the functions of the peptide or equivalent of the invention, which produces the above activities in vivo, or the functions of the DNA coding for the same, hence it is useful, for example, as an inhibitor of the secretion of certain hormones such as growth hormone, pituitary hormones (e.g. thyroid stimulating hormone, prolactin, etc.) and gastrointestinal hormones (e.g. gastrin, insulin, etc.). It can further be used as a therapeutic or prophylactic agent for

hormone-producing tumors, acromegaly, gigantism, dementia, diabetes, gastric ulcer, etc., a hormone secretion inhibitor, a tumor proliferation inhibitor, a neural activity or sleep modulator, or a like drug.

On the other hand, the oligonucleotide derivative B (antisense DNA) inhibits the functions of the peptide or equivalent of the invention, which produces the above-mentioned activities in vivo, or of the DNA coding for the same, hence is useful, for example, as a promoter of the secretion of growth hormone, pituitary hormones (e.g. thyroid stimulating hormone, prolactin, etc.), gastrointestinal hormones (e.g. gastrin, insulin, etc.) and so forth. It can further be used as a therapeutic or prophylactic agent for dwarfism, agalactia/hypogalactia, diabetes or the like, or a function modulator for digestion-related organs (e.g. a functional modulator of the stomach, small intesine, pancreas, liver, etc.) or a like drug.

For use as the above-mentioned drug, said oligonucleotide derivative or a salt thereof can be made up into pharmaceutical preparations in the same manner as the above-mentioned pharmaceutical composition containing the DNA of the invention, and can be administered to human or warm-blooded animals. For instance, said oligonucleotide derivative or a salt thereof can be administered to human or warm-blooded animals in the conventional manner either as it is or after insertion into an appropriate vector, such as a retrovirus vector, adenovirus vector or adenovirus-associated virus vector. Said oligonucleotide derivative or a salt thereof can be

administered either as it is or in the form of pharmaceutical preparations containing the same together with a physiologically acceptable carrier such as an intake-promoting auxiliary, by means of a gene gun or a catheter, e.g. a hydrogel catheter.

In the present specification and drawings, the nucleotides and amino acids, when indicated by abbreviations, are indicated by the abbreviations according to the IUPAC-IUB Commission on Biochemical Nomenclature or the abbreviations conventionally used in the relevant field of art. Examples are shown below. Where optical isomers are possible with regard to amino acids, it is the L form that is meant, unless otherwise indicated.

DNA : deoxyribonucleic acid

cDNA : complementary deoxyribonucleic acid

A : adenine

T : thymine

G : guanine

C : cytosine

RNA : ribonucleic acid

mRNA : messenger ribonucleic acid

dATP : deoxyadenosine triphosphate

dTTP : deoxythymidine triphosphate

dGTP : deoxyguanosine triphosphate

dCTP : deoxycytidine triphosphate

dNTPs : mixture of dATP, dTTP, dGTP and dCTP

ATP : adenosine triphosphate

EDTA : ethylenediaminetetraacetic acid

SDS : sodium dodecyl sulfate

EIA : enzyme immunoassay

Gly : glycine

Ala : alanine

Val : valine

Leu : leucine

Ile : isoleucine

Ser : serine

Thr : threonine

Cys : cysteine

Met : methionine

Glu : glutamic acid

Asp : aspartic acid

Lys : lysine

Arg : arginine

His : histidine

Phe : phenylalanine

Tyr : tyrosine

Trp : tryptophan

Pro : proline

Asn : asparagine

Gln : glutamine

pGlu : pyroglutamic acid

The substituents, protective groups and reagents frequently appearing in the present specification are shown below in terms of abbreviations.

BHA : benzhydrylamine

pMBHA : p-methylbenzhydrylamine

Tos : p-toluenesulfonyl

CHO : formyl

cHex : cyclohexyl

OcHex : cyclohexyl ester

Bzl : benzyl

Bom : benzyloxymethyl

Z : benzyloxycarbonyl

Br-Z : 2-bromobenzyloxycarbonyl

Boc : t-butyloxycarbonyl

DNP : dinitrophenyl

Trt : trityl

Bum : t-butoxymethyl

DCM : dichloromethane

Fmoc : N-9-fluorenylmethoxycarbonyl

HOBt : 1-hydroxybenzotriazole

HOOBt : 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazole

DCC : N,N'-dicyclohexylcarbodiimide

TFA : trifluoroacetic acid

DIEA : diisopropylethylamine

In the present specification, the sequence identifier numbers in the sequence listing respectively refer to the following.

[SEQ ID NO:1]

The amino acid sequence of from the 89th residue to the 105th residue in the amino acid sequence shown in Fig. 2.

[SEQ ID NO:2]

The amino acid sequence of from the 91st residue to the 105th residue in the amino acid sequence shown in Fig. 2.

[SEQ ID NO:3]

The amino acid sequence of from the 77th residue to the 105th residue in the amino acid sequence shown in Fig. 2.

[SEQ ID NO:4]

The amino acid sequence of from the 44th residue to the 105th residue in the amino acid sequence shown in Fig. 2.

[SEQ ID NO:5]

The amino acid sequence of from the 21st residue to the 105th residue in the amino acid sequence shown in Fig. 2.

[SEQ ID NO:6]

The amino acid sequence of from the 1st residue to the 105th residue in the amino acid sequence shown in Fig. 2.

[SEQ ID NO:7]

A nucleotide sequence of from the 268th to the 318th nucleotide in the nucleotide sequence shown in Fig. 2.

[SEQ ID NO:8]

A nucleotide sequence of from the 274th to the 318th nucleotide in the nucleotide sequence shown in Fig. 2.

[SEQ ID NO:9]

A nucleotide sequence of from the 232nd to the 318th nucleotide in the nucleotide sequence shown in Fig. 2.

[SEQ ID NO:10]

A nucleotide sequence of from the 133rd to the 318th nucleotide

in the nucleotide sequence shown in Fig. 2.

[SEQ ID NO:11]

A nucleotide sequence of from the 64th to the 318th nucleotide in the nucleotide sequence shown in Fig. 2.

[SEQ ID NO:12]

A nucleotide sequence of from the 4th to the 318th nucleotide in the nucleotide sequence shown in Fig. 2.

[SEQ ID NO:13]

The amino acid sequence of from the 77th residue to the 88th residue in the amino acid sequence shown in Fig. 2.

[SEQ ID NO:14]

The amino acid sequence of from the 44th residue to the 76th residue in the amino acid sequence shown in Fig. 2.

[SEQ ID NO:15]

The amino acid sequence of from the 21st residue to the 43rd residue in the amino acid sequence shown in Fig. 2.

[SEQ ID NO:16]

The amino acid sequence of from the 1st residue to the 20th residue in the amino acid sequence shown in Fig. 2.

[SEQ ID NO:17]

A nucleotide sequence of from the 232nd to the 267th nucleotide in the nucleotide sequence shown in Fig. 2.

[SEQ ID NO:18]

A nucleotide sequence of from the 133rd to the 231st nucleotide in the nucleotide sequence shown in Fig. 2.

[SEQ ID NO:19]

A nucleotide sequence of from the 64th to the 132nd nucleotide in the nucleotide sequence shown in Fig. 2.

[SEQ ID NO:20]

A nucleotide sequence of from the 4th to the 63rd nucleotide in the nucleotide sequence shown in Fig. 2.

[SEQ ID NO:21]

The amino acid sequence of from the 1st residue to the 88th residue in the amino acid sequence shown in Fig. 2.

[SEQ ID NO:22]

The amino acid sequence of the known rat coetistatin.

[SEQ ID NO:23]

The amino acid sequence of the known rat somotostatin.

[SEQ ID NO:24]

The nucleotide sequence of a primer used for the cloning of a DNA coding for a peptide of the invention.

[SEQ ID NO:25]

The nucleotide sequence of a primer used for the cloning of a DNA coding for a peptide of the invention.

The transformant Escherichia coli JM109/phCSP6 obtained in Example 2 mentioned later herein has been deposited with the Ministry of International Trade and Industry National Institute of Bioscience and Human Technology (NIBH) since June 6, 1996 under the accession number FERM BP-5564 and with the Institute for Fermentation, Osaka (IFO) since June 5, 1996 under the accession number IFO 15967.

The following examples illustrate the present invention in further detail. They are, however, by no means limitative of the

scope of the present invention. Those gene manipulation procedures described in Molecular Cloning were followed in genetically manipulating Escherichia coli.

Example 1

Synthesis of cDNA from a human brain poly(A)$^+$ RNA fraction and amplification of a physiologically active peptide cDNA by the RT-PCR technique

To 5 μg of a human brain poly(A)$^+$ RNA fraction purchased from Clontech was added a random DNA hexamer mixture (BRL) as the primer, and complementary DNA synthesis was carried out using Moloney murine leukemia virus-derived reverse transcriptase (BRL) and the buffer attached thereto. After the reaction, the product was extracted with phenol-chloroform (1:1) and precipitated with ethanol, and the precipitate was dissolved in 30 μl of TE (10 mM Tris-HCl (pH 7.5), 1 mM EDTA). Using 1 μl of the thus-prepared cDNA as a template, amplification was carried out by PCR using the following two primers:

5'-ACAAGATGCCATTGTCCCCCGGCCTCCT-3' (SEQ ID NO:35)

5'-TTCAGGTCTGTAATTAAACTTGCGTGA-3' (SEQ ID NO:36)

The composition of the reaction mixture was as follows: synthetic DNA primers (5' primer sequence and 3' primer sequence) 10 pM each, 0.25 mM dNTPs, Ex Taq DNA polymerase 0.5 μl and the buffer attached to the enzyme 10 μl, the total reaction mixture amounting to 100 μl. Amplification was carried out, using a Thermal Cycler apparatus (Perkin Elmer), in 35 cycles each comprising: 30 seconds at 95℃, 1 minute at 65℃ and 30 seconds at 72℃. The amplification product was identified by 1.2% agarose electrophoresis

and ethidium bromide staining.

Example 2

Subcloning of the PCR product into a plasmid vector and selection of a novel physiologically active peptide candidate clone

The reaction product after the PCR carried out in Example 1 was separated using a 1.2% agarose gel, the band was excised with a razor and, then, the DNA was recovered by SUPRECOI™ (Takara) treatment, phenol extraction and ethanol extraction. The DNA recovered was subcloned into the plasmid vector pCR™II according to the prescription of TA Cloning Kit (Invitogen). This was introduced into Escherichia coli JM109 competent cells (Takara Shuzo) and, thereafter, clones having the cDNA insert fragment were selected in LB agar medium containing ampicillin, IPTG and X-gal and, by isolating a white-colored clone alone using a sterile toothpick, whereby a transformant, Escherichia coli JM109/phCSP6, was obtained.

This clone was cultured overnight in ampicillin-containing LB medium and a plasmid DNA was prepared using an automated plasmid extractor (Kurabo). A portion of the DNA prepared was cleaved with EcoRI and the size of the cDNA fragment inserted therein was confirmed. Another portion of the remaining DNA was further subjected to RNase treatment, phenol/chloroform extraction and ethanol precipitation for the purpose of concentration. The reaction for nucleotide sequence determination was carried out using a DyeDeoxy Terminator Cycle Sequencing Kit (ABI) and decoding was carried out using a fluorescence-based automated sequencer. The nucleotide sequence information obtained was processed using a DNA SIS system (Hitachi

System Engineering). The nucleotide sequence thus determined is shown in Fig. 1.

Based on the nucleotide sequence determined (Fig. 1), homology searching was carried out and, as a result, it was found that the cDNA fragment inserted into the plasmid harbored by the transformant Escherichia coli JM109/phCSP6 codes for a novel physiologically active peptide. Furthermore, for confirming that fact, the nucleotide sequence was converted to an amino acid sequence using a DNASIS system (Hitachi System Engineering) (Fig. 2), followed by homology searching based on hydrophobicity plotting (Fig. 4) and on the amino acid sequence, whereupon homology with rat cortistatin (U51919) and rat somatostatin (J00788) was found (Fig. 5).

The abbreviations in the above parentheses are serial numbers given on the occasion of registration of data thereon with the NBRF-PIR and generally referred to as accession numbers.

[Industrial Applicability]

The peptides and precursors thereof, inclusive of salts thereof, of the present invention have somatostatin-like or cortistatin-like activities, such as (1) growth hormone secretion inhibiting activity, (2) inhibitory activity against the secretion of pituitary hormones such as thyroid stimulating hormone and prolactin, (3) inhibitory activity against the secretion of digestive tract hormones such as gastrin and insulin, (4) neurotransmitter activity, (5) cell proliferation activity, (6) inhibitory activity against the activities of acetylcholine, which is a REM sleep inducer, and so on. Therefore, the peptides, precursors and salts of the

invention are useful as drugs, for example as therapeutic or prophylactic agents for hormone-producing tumors, acromegaly, gigantism, dementia, diabetes, gastric ulcer and the like, hormone secretion inhibitors, tumor growth inhibitors, neural activity or sleep modulators and so forth.

The DNAs coding for the peptide or precursor of the invention are useful, for example, as agents for the gene therapy or prevention of hormone-producing tumors, acromegaly, gigantism, dementia, diabetes, gastric ulcer and the like, hormone secretion inhibitors, tumor growth inhibitors, neural activity or sleep modulators and so forth. Furthermore, the DNAs of the invention are useful as agents for the gene diagnosis of diseases such as, for example, hormone-producing tumors, acromegaly, gigantism, dementia, diabetes, gastric ulcer and the like.

The antibodies against the peptide, precursor or salt of the invention can specifically recognize the peptide, precursor or salt of the invention, hence can be used for assaying the peptide or equivalent of the invention in test solutions.

The peptides, precursors or salts of the invention are useful as reagents for screening for compounds, or salts thereof, capable of modifying the binding of the peptides, precursors or salts of the invention to the receptors.

[Sequence Listing]

SEQ ID NO:1

SEQUENCE CHARACTERISTICA

LENGTH OF THE SEQUENCE: 17

TYPE OF THE SEQUENCE: Amino acid

TOPOLOGY: Linear

MOLECULE TYPE OF THE SEQUENCE: Peptide

SEQUENCE

Asp Arg Met Pro Cys Arg Asn Phe Phe Trp Lys Thr Phe Ser Ser Cys

1 5 10 15

Lys

SEQ ID NO:2

SEQUENCE CHARACTERISTICA

LENGTH OF THE SEQUENCE: 15

TYPE OF THE SEQUENCE: Amino acid

TOPOLOGY: Linear

MOLECULE TYPE OF THE SEQUENCE: Peptide

SEQUENCE

Met Pro Cys Arg Asn Phe Phe Trp Lys Thr Phe Ser Ser Cys Lys

1 5 10 15

SEQ ID NO:3

SEQUENCE CHARACTERISTICA

LENGTH OF THE SEQUENCE: 29

TYPE OF THE SEQUENCE: Amino acid

TOPOLOGY: Linear

MOLECULE TYPE OF THE SEQUENCE: Peptide

SEQUENCE

Gln Glu Gly Ala Pro Pro Gln Gln Ser Ala Arg Arg Asp Arg Met Pro

1 5 10 15

Cys Arg Asn Phe Phe Trp Lys Thr Phe Ser Ser Cys Lys

20 25

SEQ ID NO:4

SEQUENCE CHARACTERISTICA

LENGTH OF THE SEQUENCE: 62

TYPE OF THE SEQUENCE: Amino acid

TOPOLOGY: Linear

MOLECULE TYPE OF THE SEQUENCE: Peptide

SEQUENCE

Ser Ser Leu Leu Thr Phe Leu Ala Trp Trp Phe Glu Trp Thr Ser Gln

1 5 10 15

Ala Ser Ala Gly Pro Leu Ile Gly Glu Glu Ala Arg Glu Val Ala Arg

20 25 30

Arg Gln Glu Gly Ala Pro Pro Gln Gln Ser Ala Arg Arg Asp Arg Met

35 40 45

Pro Cys Arg Asn Phe Phe Trp Lys Thr Phe Ser Ser Cys Lys

50 55 60

SEQ ID NO:5

SEQUENCE CHARACTERISTICA

LENGTH OF THE SEQUENCE: 85

TYPE OF THE SEQUENCE: Amino acid

TOPOLOGY: Linear

MOLECULE TYPE OF THE SEQUENCE: Peptide

SEQUENCE

Leu Pro Leu Glu Gly Gly Pro Thr Gly Arg Asp Ser Glu His Met Gln
1 5 10 15

Glu Ala Ala Gly Ile Arg Lys Ser Ser Leu Leu Thr Phe Leu Ala Trp
20 25 30

Trp Phe Glu Trp Thr Ser Gln Ala Ser Ala Gly Pro Leu Ile Gly Glu
35 40 45

Glu Ala Arg Glu Val Ala Arg Arg Gln Glu Gly Ala Pro Pro Gln Gln
50 55 60

Ser Ala Arg Arg Asp Arg Met Pro Cys Arg Asn Phe Phe Trp Lys Thr
65 70 75 80

Phe Ser Ser Cys Lys
85

SEQ ID NO:6

SEQUENCE CHARACTERISTICA

LENGTH OF THE SEQUENCE: 105

TYPE OF THE SEQUENCE: Amino acid

TOPOLOGY: Linear

MOLECULE TYPE OF THE SEQUENCE: Peptide

SEQUENCE

Met Pro Leu Ser Pro Gly Leu Leu Leu Leu Leu Ser Gly Ala Thr
1 5 10 15

Ala Thr Ala Ala Leu Pro Leu Glu Gly Gly Pro Thr Gly Arg Asp Ser
20 25 30

Glu His Met Gln Glu Ala Ala Gly Ile Arg Lys Ser Ser Leu Leu Thr

35 40 45

Phe Leu Ala Trp Trp Phe Glu Trp Thr Ser Gln Ala Ser Ala Gly Pro

50 55 60

Leu Ile Gly Glu Glu Ala Arg Glu Val Ala Arg Arg Gln Glu Gly Ala

65 70 75 80

Pro Pro Gln Gln Ser Ala Arg Arg Asp Arg Met Pro Cys Arg Asn Phe

85 90 95

Phe Trp Lys Thr Phe Ser Ser Cys Lys

100 105

SEQ ID NO:7

LENGTH OF THE SEQUENCE: 51

TYPE OF THE SEQUENCE: Nucleic acid

STRANDEDNESS: Double

TOPOLOGY: Linear

MOLECULE TYPE: cDNA

SEQUENCE

GACAGAATGC CCTGCAGGAA CTTCTTCTGG AAGACCTTCT CCTCCTGCAA A 51

SEQ ID NO:8

LENGTH OF THE SEQUENCE: 45

TYPE OF THE SEQUENCE: Nucleic acid

STRANDEDNESS: Double

TOPOLOGY: Linear

MOLECULE TYPE: cDNA

SEQUENCE

ATGCCCTGCA GGAACTTCTT CTGGAAGACC TTCTCCTCCT GCAAA 45

SEQ ID NO:9

LENGTH OF THE SEQUENCE: 87

TYPE OF THE SEQUENCE: Nucleic acid

STRANDEDNESS: Double

TOPOLOGY: Linear

MOLECULE TYPE: cDNA

SEQUENCE

CAGGAAGGCG CACCCCCCCA GCAATCCGCG CGCCGGGACA GAATGCCCTG CAGGAACTTC 60

TTCTGGAAGA CCTTCTCCTC CTGCAAA 87

SEQ ID NO:10

LENGTH OF THE SEQUENCE: 186

TYPE OF THE SEQUENCE: Nucleic acid

STRANDEDNESS: Double

TOPOLOGY: Linear

MOLECULE TYPE: cDNA

SEQUENCE

AGCAGCCTCC TGACTTTCCT CGCTTGGTGG TTTGAGTGGA CCTCCCAGGC CAGTGCCGGG 60

CCCCTCATAG GAGAGGAAGC TCGGGAGGTG GCCAGGCGGC AGGAAGGCGC ACCCCCCCAG 120

CAATCCGCGC GCCGGGACAG AATGCCCTGC AGGAACTTCT TCTGGAAGAC CTTCTCCTCC 180

TGCAAA 186

SEQ ID NO:11

LENGTH OF THE SEQUENCE: 255

TYPE OF THE SEQUENCE: Nucleic acid

STRANDEDNESS: Double

TOPOLOGY: Linear

MOLECULE TYPE: cDNA

SEQUENCE

CTGCCCCTGG AGGGTGGCCC CACCGGCCGA GACAGCGAGC ATATGCAGGA AGCGGCAGGA 60

ATAAGGAAAA GCAGCCTCCT GACTTTCCTC GCTTGGTGGT TTGAGTGGAC CTCCCAGGCC 120

AGTGCCGGGC CCCTCATAGG AGAGGAAGCT CGGGAGGTGG CCAGGCGGCA GGAAGGCGCA 180

CCCCCCCAGC AATCTGCGCG CCGGGACAGA ATGCCCTGCA GGAACTTCTT CTGGAAGACC 240

TTCTCCTCCT GCAAA 255

SEQ ID NO:12

LENGTH OF THE SEQUENCE: 315

TYPE OF THE SEQUENCE: Nucleic acid

STRANDEDNESS: Double

TOPOLOGY: Linear

MOLECULE TYPE: cDNA

SEQUENCE

ATGCCATTGT CCCCCGGCCT CCTGCTGCTG CTGCTCTCCG GGGCCACGGC CACCGCTGCC 60

CTGCCCCTGG AGGGTGGCCC CACCGGCCGA GACAGCGAGC ATATGCAGGA AGCGGCAGGA 120

ATAAGGAAAA GCAGCCTCCT GACTTTCCTC GCTTGGTGGT TTGAGTGGAC CTCCCAGGCC 180

AGTGCCGGGC CCCTCATAGG AGAGGAAGCT CGGGAGGTGG CCAGGCGGCA GGAAGGCGCA 240

CCCCCCCAGC AATCCGCGCG CCGGGACAGA ATGCCCTGCA GGAACTTCTT CTGGAAGACC 300

TTCTCCTCCT GCAAA 315

SEQ ID NO:13

SEQUENCE CHARACTERISTICA

LENGTH OF THE SEQUENCE: 12

TYPE OF THE SEQUENCE: Amino acid

TOPOLOGY: Linear

MOLECULE TYPE OF THE SEQUENCE: Peptide

SEQUENCE

Gln Glu Gly Ala Pro Pro Gln Gln Ser Ala Arg Arg

1 5 10

SEQ ID NO:14

SEQUENCE CHARACTERISTICA

LENGTH OF THE SEQUENCE: 33

TYPE OF THE SEQUENCE: Amino acid

TOPOLOGY: Linear

MOLECULE TYPE OF THE SEQUENCE: Peptide

SEQUENCE

Ser Ser Leu Leu Thr Phe Leu Ala Trp Trp Phe Glu Trp Thr Ser Gln

1 5 10 15

Ala Ser Ala Gly Pro Leu Ile Gly Glu Glu Ala Arg Glu Val Ala Arg

20 25 30

Arg

SEQ ID NO:15

SEQUENCE CHARACTERISTICA

LENGTH OF THE SEQUENCE: 23

TYPE OF THE SEQUENCE: Amino acid

TOPOLOGY: Linear

MOLECULE TYPE OF THE SEQUENCE: Peptide

SEQUENCE

Leu Pro Leu Glu Gly Gly Pro Thr Gly Arg Asp Ser Glu His Met Gln
1 5 10 15

Glu Ala Ala Gly Ile Arg Lys
20

SEQ ID NO:16

SEQUENCE CHARACTERISTICA

LENGTH OF THE SEQUENCE: 20

TYPE OF THE SEQUENCE: Amino acid

TOPOLOGY: Linear

MOLECULE TYPE OF THE SEQUENCE: Peptide

SEQUENCE

Met Pro Leu Ser Pro Gly Leu Leu Leu Leu Leu Ser Gly Ala Thr
1 5 10 15

Ala Thr Ala Ala
20

SEQ ID NO:17

LENGTH OF THE SEQUENCE: 36

TYPE OF THE SEQUENCE: Nucleic acid

STRANDEDNESS: Double

TOPOLOGY: Linear

MOLECULE TYPE: cDNA

SEQUENCE

CAGGAAGGCG CACCCCCCCA GCAATCTGCG CGCCGG 36

SEQ ID NO:18

LENGTH OF THE SEQUENCE: 99

TYPE OF THE SEQUENCE: Nucleic acid

STRANDEDNESS: Double

TOPOLOGY: Linear

MOLECULE TYPE: cDNA

SEQUENCE

AGCAGCCTCC TGACTTTCCT CGCTTGGTGG TTTGAGTGGA CCTCCCAGGC CAGTGCCGGG

60

CCCCTCATAG GAGAGGAAGC TCGGGAGGTG GCCAGGCGG 99

SEQ ID NO:19

LENGTH OF THE SEQUENCE: 69

TYPE OF THE SEQUENCE: Nucleic acid

STRANDEDNESS: Double

TOPOLOGY: Linear

MOLECULE TYPE: cDNA

SEQUENCE

CTGCCCCTGG AGGGTGGCCC CACCGGCCGA GACAGCGAGC ATATGCAGGA AGCGGCAGGA

60

ATAAGGAAA 69

SEQ ID NO:20

LENGTH OF THE SEQUENCE: 60

TYPE OF THE SEQUENCE: Nucleic acid

STRANDEDNESS: Double

TOPOLOGY: Linear

MOLECULE TYPE: cDNA

SEQUENCE

ATGCCATTGT CCCCCGGCCT CCTGCTGCTG CTGCTCTCCG GGGCCACGGC CACCGCTGCC 60

SEQ ID NO:21

SEQUENCE CHARACTERISTICA

LENGTH OF THE SEQUENCE: 88

TYPE OF THE SEQUENCE: Amino acid

TOPOLOGY: Linear

MOLECULE TYPE OF THE SEQUENCE: Peptide

SEQUENCE

Met Pro Leu Ser Pro Gly Leu Leu Leu Leu Leu Ser Gly Ala Thr
1 5 10 15

Ala Thr Ala Ala Leu Pro Leu Glu Gly Gly Pro Thr Gly Arg Asp Ser
20 25 30

Glu His Met Gln Glu Ala Ala Gly Ile Arg Lys Ser Ser Leu Leu Thr
35 40 45

Phe Leu Ala Trp Trp Phe Glu Trp Thr Ser Gln Ala Ser Ala Gly Pro
50 55 60

Leu Ile Gly Glu Glu Ala Arg Glu Val Ala Arg Arg Gln Glu Gly Ala
65 70 75 80

Pro Pro Gln Gln Ser Ala Arg Arg
85

SEQ ID NO:22

SEQUENCE CHARACTERISTICA

LENGTH OF THE SEQUENCE: 15

TYPE OF THE SEQUENCE: Amino acid

TOPOLOGY: Linear

MOLECULE TYPE OF THE SEQUENCE: Peptide

SEQUENCE

Lys Pro Cys Lys Asn Phe Phe Trp Lys Thr Phe Ser Ser Lys Cys

1 5 10 15

SEQ ID NO:23

SEQUENCE CHARACTERISTICA

LENGTH OF THE SEQUENCE: 14

TYPE OF THE SEQUENCE: Amino acid

TOPOLOGY: Linear

MOLECULE TYPE OF THE SEQUENCE: Peptide

SEQUENCE

Ala Gly Cys Lys Asn Phe Phe Trp Lys Thr Phe Thr Ser Cys

1 5 10

SEQ ID NO:24

LENGTH OF THE SEQUENCE: 28

TYPE OF THE SEQUENCE: Nucleic acid

STRANDEDNESS: Single

TOPOLOGY: Linear

MOLECULE TYPE: Synthetic DNA

SEQUENCE

ACAAGATGCC ATTGTCCCCC GGCCTCCT 28

SEQ ID NO:25

LENGTH OF THE SEQUENCE: 27

TYPE OF THE SEQUENCE: Nucleic acid

STRANDEDNESS: Single

TOPOLOGY: Linear

MOLECULE TYPE: Synthetic DNA

SEQUENCE

TTCAGGTCTG TAATTAAACT TGCGTGA 27

[Brief Description of the Drawings]

Fig. 1 shows the nucleotide sequence of the DNA obtained in Example 2 and coding for the peptide hCS-17 of the present invention and a precursor thereof.

Fig. 2 shows the nucleotide sequence of the DNA obtained in Example 2 and coding for the peptide hCS-17 of the invention and a precursor thereof, and the amino acid sequence deduced therefrom.

Fig. 3 shows the nucleotide sequence of the DNA coding for the peptide hCS-17 of the invention and a precursor thereof, and the amino acid sequence deduced therefrom. In Fig. 2, the codon coding for the 85th amino acid serine in TCC while, in Fig. 3, the corresponding codon is TCT.

Fig. 4 shows the results of hydrophobicity plotting analysis of the amino acid sequence of the precursor of the invention shown in Fig. 2.

[Document] Figure

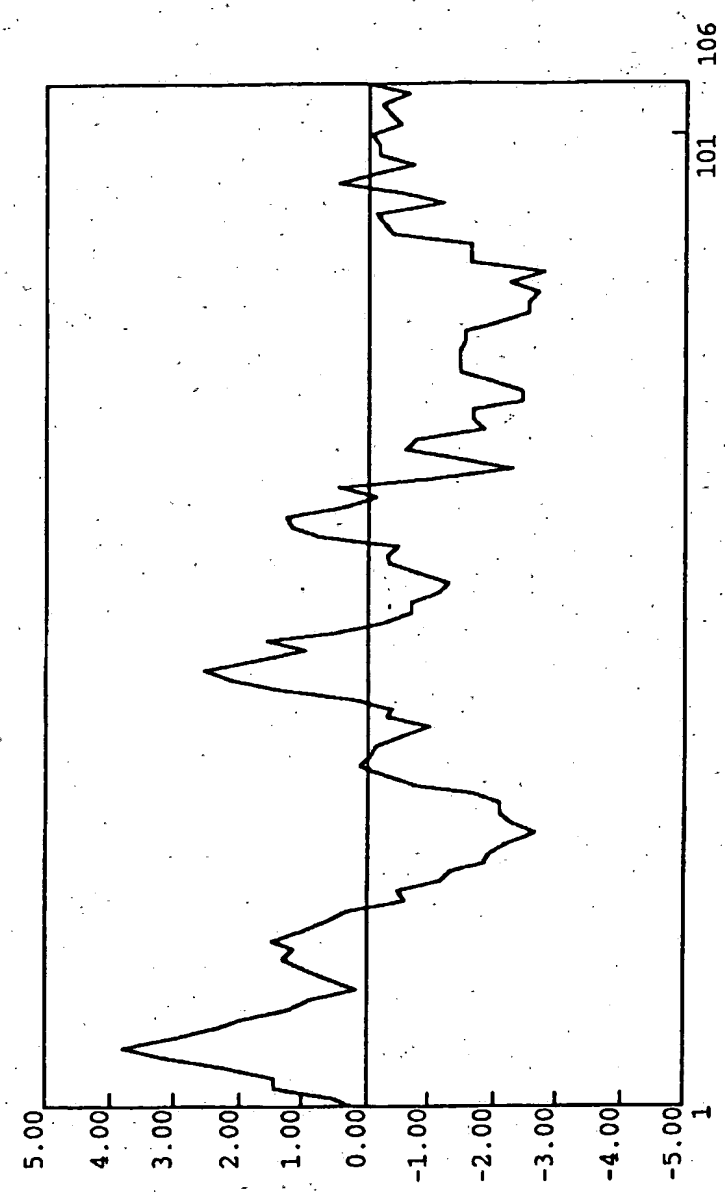
[Figure 1]

```
        10          20          30          40          50          60
ACAAGATGCC ATTGTCCCCC GGCCTCCTGC TGCTGCTGCT CTCCGGGGCC ACGGCCACCG

        70          80          90          100         110         120
CTGCCCTGCC CCTGGAGGGT GGCCCCACCG GCCGAGACAG CGAGCATATG CAGGAAGCGG

       130         140         150         160         170         180
CAGGAATAAG GAAAAGCAGC CTCCTGACTT TCCTCGCTTG GTGGTTTGAG TGGACCTCCC

       190         200         210         220         230         240
AGGCCAGTGC CGGGCCCCTC ATAGGAGAGG AAGCTCGGGA GGTGGCCAGG CGGCAGGAAG

       250         260         270         280         290         300
GCGCACCCCC CCAGCAATCC GCGCGCCGGG ACAGAATGCC CTGCAGGAAC TTCTTCTGGA

       310         320         330         340         350         360
AGACCTTCTC CTCCTGCAAA TAAAACCTCA CCCATGAATG CTCACGCAAG TTTAATTACA

       370         380         390         400         410         420
GACCTGAA.. .......... .......... .......... .......... ..........
```

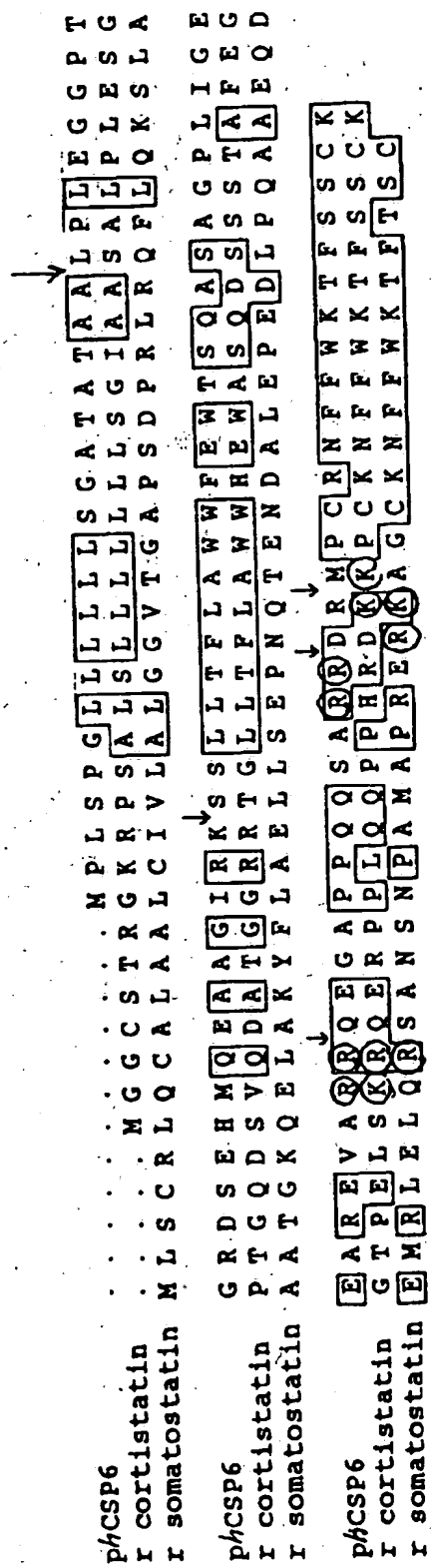
[Figure 2]

```
  3 AAGATGCCATTGTCCCCCGGCCTCCTGCTGCTGCTGCTCTCCGGGGCCACGGCCACCGCT    62
  1    MetProLeuSerProGlyLeuLeuLeuLeuLeuSerGlyAlaThrAlaThrAla      19

 63 GCCCTGCCCCTGGAGGGTGGCCCCACCGGCCGAGACAGCGAGCATATGCAGGAAGCGGCA   122
 19 AlaLeuProLeuGluGlyGlyProThrGlyArgAspSerGluHisMetGlnGluAlaAla    39

123 GGAATAAGGAAAAGCAGCCTCCTGACTTTCCTCGCTTGGTGGTTTGAGTGGACCTCCCAG   182
 39 GlyIleArgLysSerSerLeuLeuThrPheLeuAlaTrpTrpPheGluTrpThrSerGln    59

183 GCCAGTGCCGGGCCCCTCATAGGAGAGGAAGCTCGGGAGGTGGCCAGGCGGCAGGAAGGC   242
 59 AlaSerAlaGlyProLeuIleGlyGluGluAlaArgGluValAlaArgArgGlnGluGly    79

243 GCACCCCCCCAGCAATCCGCGCGCCGGGACAGAATGCCCTGCAGGAACTTCTTCTGGAAG   302
 79 AlaProProGlnGlnSerAlaArgArgAspArgMetProCysArgAsnPhePheTrpLys    99

303 ACCTTCTCCTCCTGCAAATAAAACCTCACCCATGAATGCTCACGCAAGTTTAATTACAGA   362
 99 ThrPheSerSerCysLys***                                         106

363 CCTGAA                                                        368
106                                                               106
```

【 Figure 3 】



【 Figure 4 】


phCSP6
r cortistatin
r somatostatin
phCSP6
r cortistatin
r somatostatin
phCSP6
r cortistatin
r somatostatin

[Document Name] Abstract

[Abstract]

[Problems] Provision of novel physiologically active peptides.

[Means for solve] The peptide comprising an amino acid sequence represented by SEQ ID NO: 1, a precursor thereof or a salt thereof, a DNA cording for the peptide, a recombinant vector, a transformant, a method of producing the peptide, a pharmaceutical composition comprising the peptide, an antibody against the peptide, a method of screening for an antagonist or an agonist against a receptor and a kit for the screening.

[Effect] The peptides and precursors thereof, inclusive salts thereof, of the present invention are useful as a pharmaceutical composition, for example as therapeutic or prophylactic agents for hormone-producing tumors, acromegaly, gigantism, dementia, gastric ulcer and the like, hormone secretion inhibitors, tumor growth inhibitors, neural activity or sleep modulators, etc. The DNAs coding for the peptides or precursors of the invention are useful as a pharmaceutical composition, for example as agents for the gene therapy or prevention of hormone-producing tumors, acromegaly, gigantism, dementia, gastric ulcer and the like, hormone secretion inhibitors, tumor growth inhibitors, neural activity or sleep modulators, etc. Furthermore, the DNAs coding for the peptides or precursors of the invention are useful as agents for the gene diagnosis of various diseases, for example, hormone-producing tumors, acromegaly, gigantism, dementia, gastric ulcer, etc. The antibodies against the peptides, precursors or salts of the invention can be

used for assaying the peptides, precursors or salts of the invention in test solutions. The peptides, precursors or salts of the invention are useful as reagents for screening for compounds, or salts thereof, capable of modifying the binding of the peptides, precursors or salts of the invention to certain receptors.

[Selected figure] None